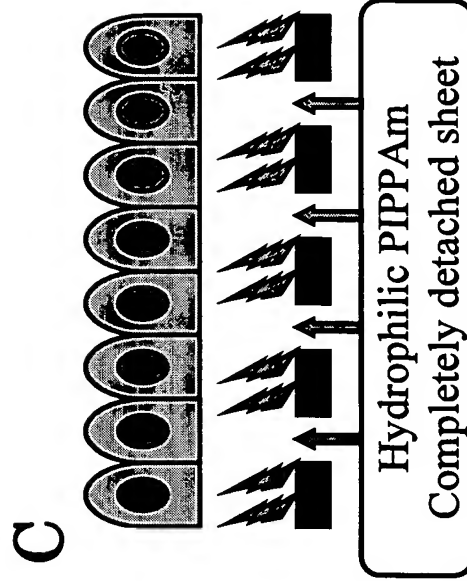
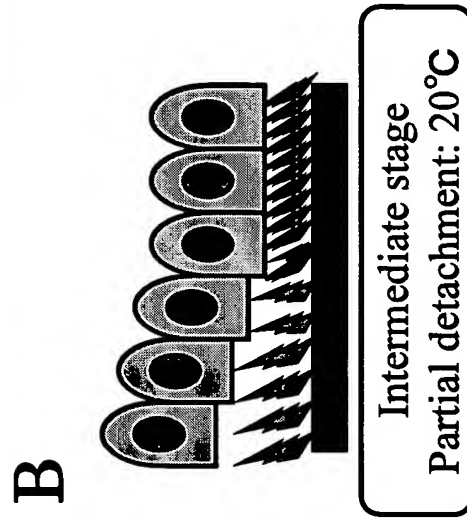
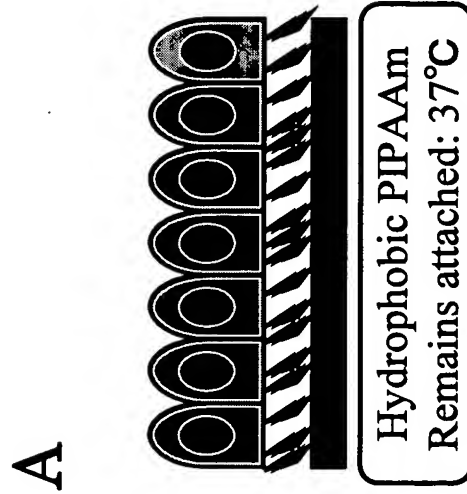


FIG. 1A

Cell Sheet Constructs



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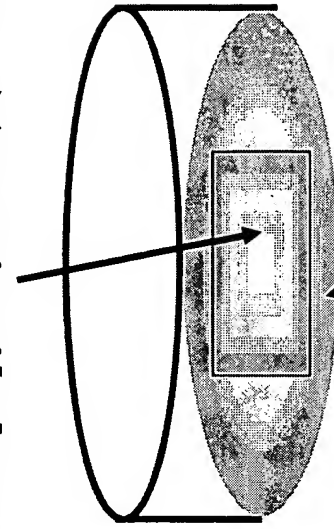
Exemplary Dimensions:

$1.11 \pm 0.05 \text{ cm}^2$ in area

$50.2 \pm 6.0 \mu\text{m}$ thick

Temperature responsive polymer

Poly (N-isopropylacrylamide) (PIPAAm)



Poly (acrylamide) (PAAm)

Temperature responsive culture dish

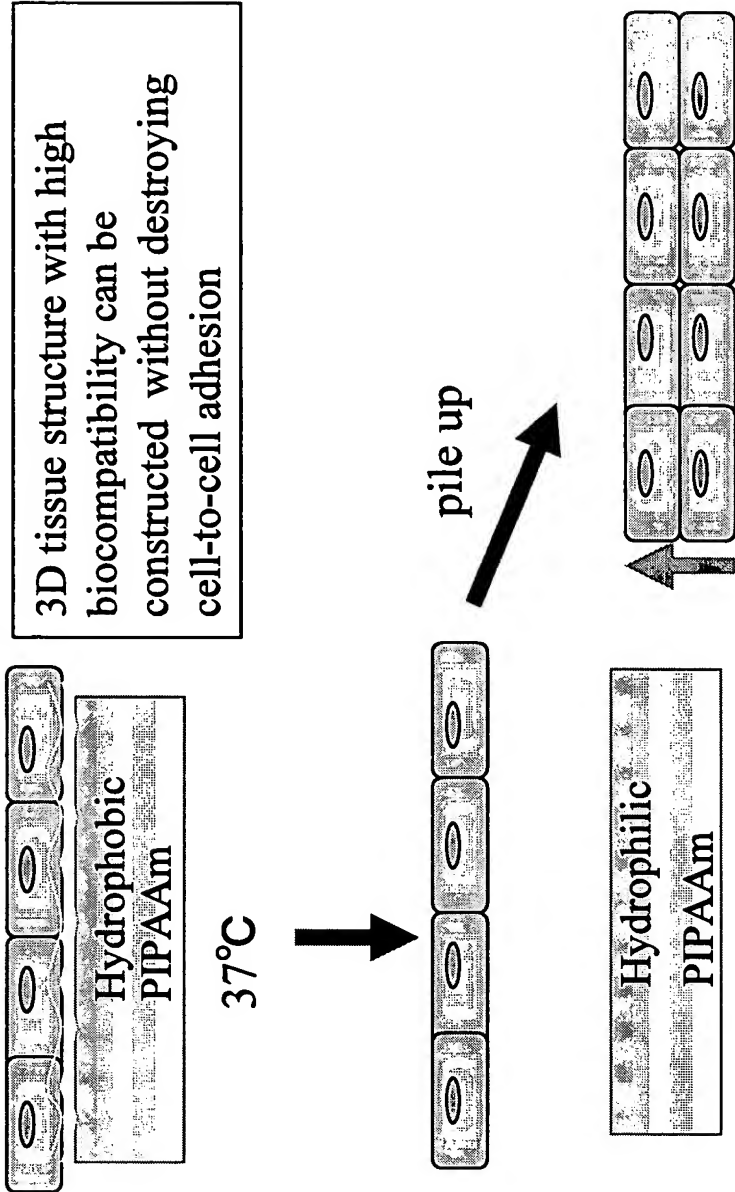


FIG.1B

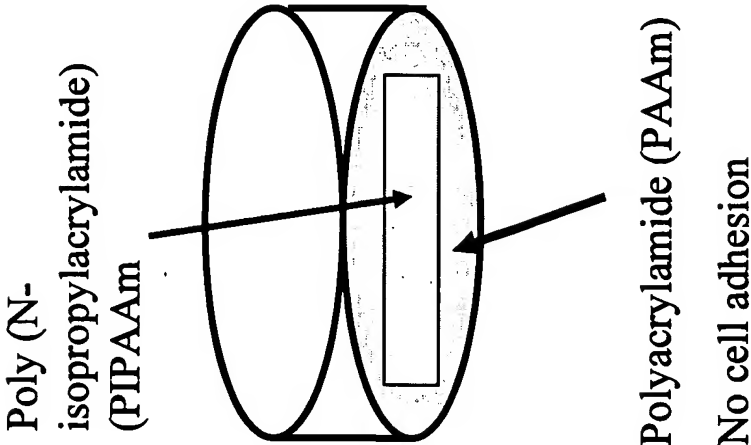
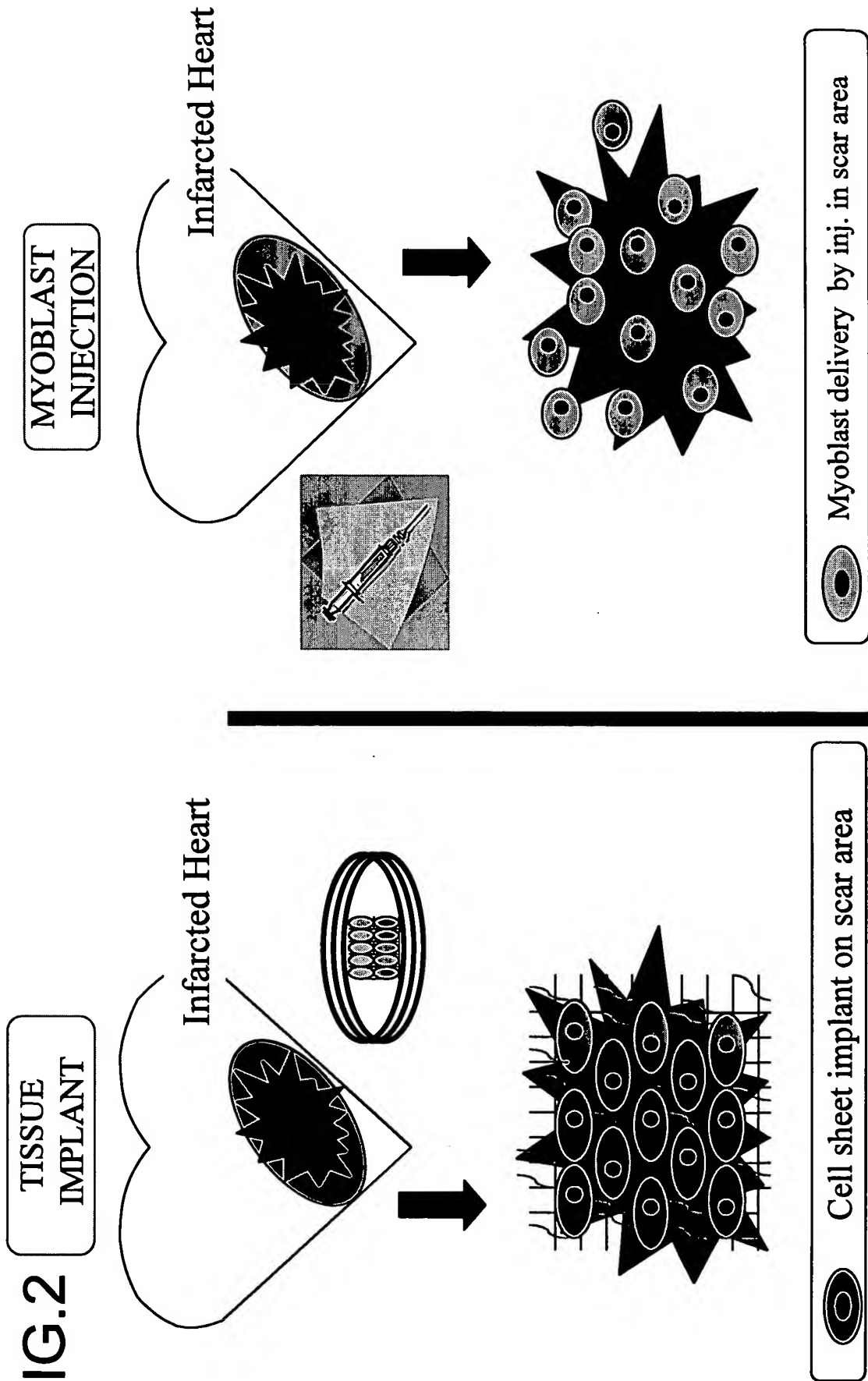


FIG.2



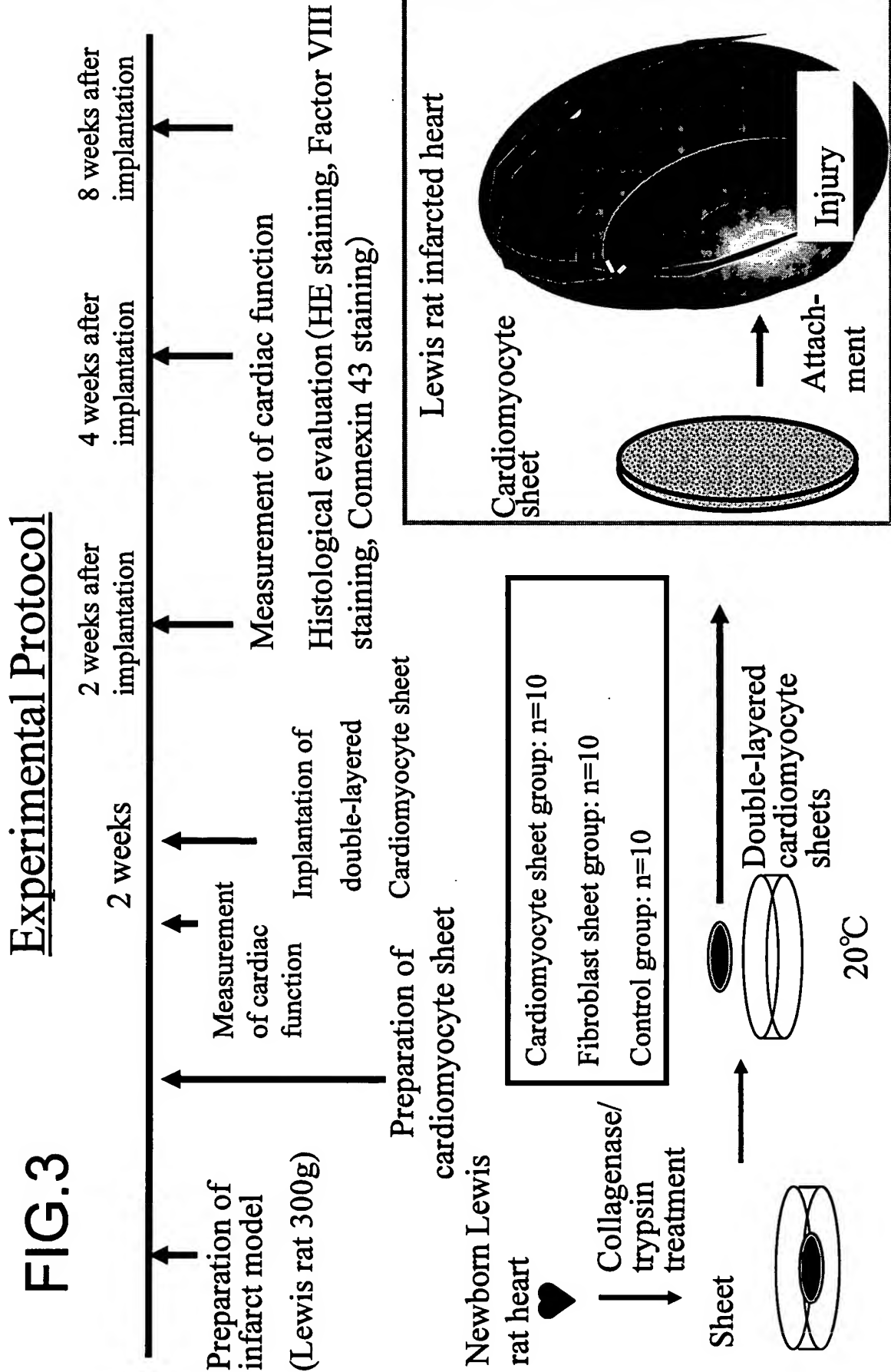


FIG.4 Regenerative therapy for cardiac
muscle by cell transplantation

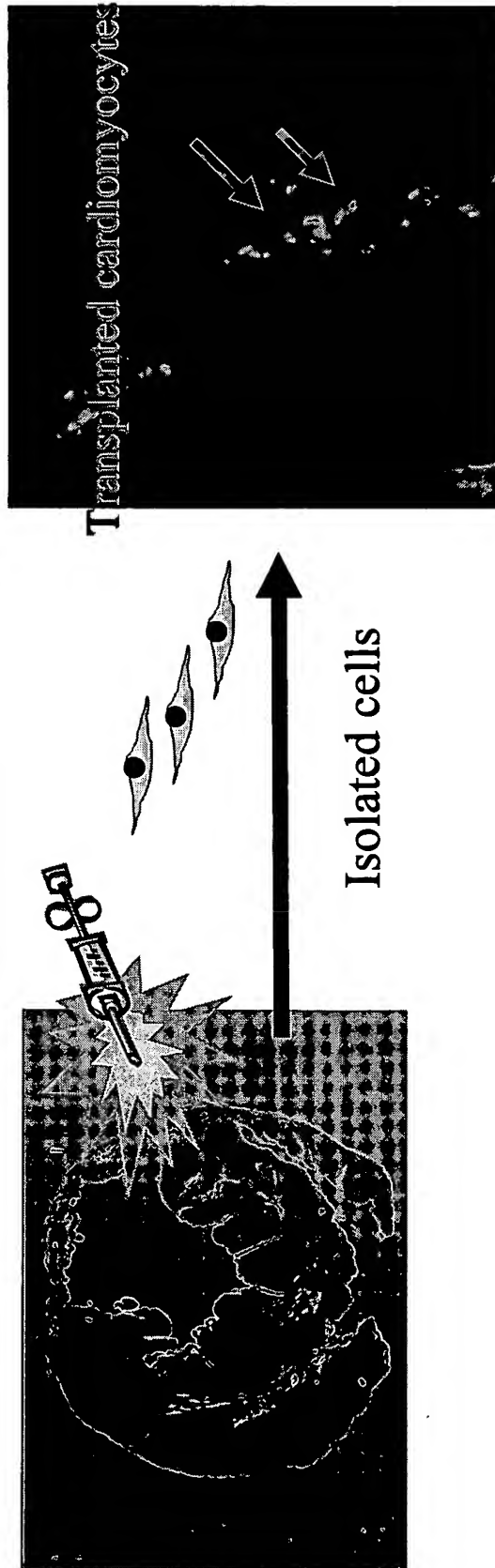


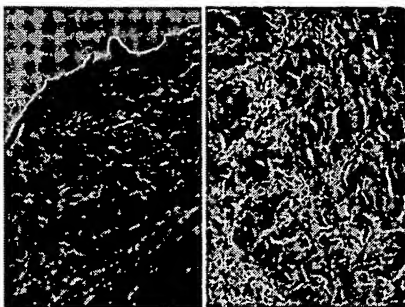
FIG.5 Problems with tissue transplantation

Cardiac muscle graft with scaffold

Alignment and cell-to-cell adhesion of transplanted cells within scaffold

Changes in scaffold in organism : elicitation of inflammation

Acceptance of scaffold by recipient's heart



Development of high biocompatible cardiac muscle graft without scaffold

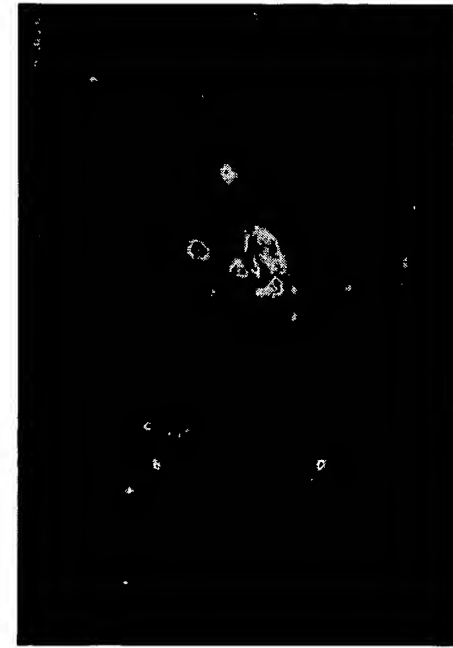
FIG.6 Implantation of cardiomyocyte sheet into infarcted heart



Cardiomyocyte sheet

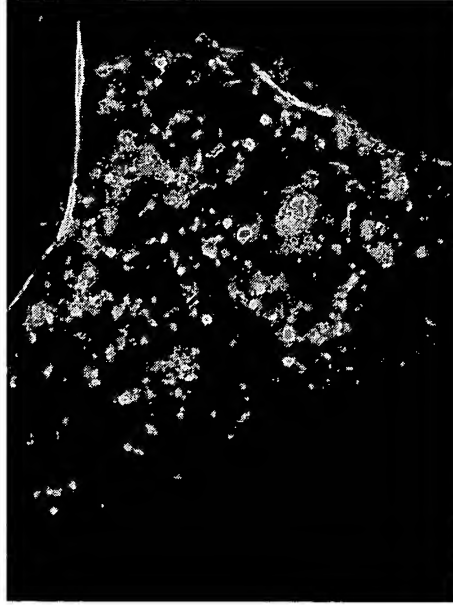


Implantation into rat infarct model



In vitro

Implantation of GFP rat newborn cardiomyocyte sheet



In vivo

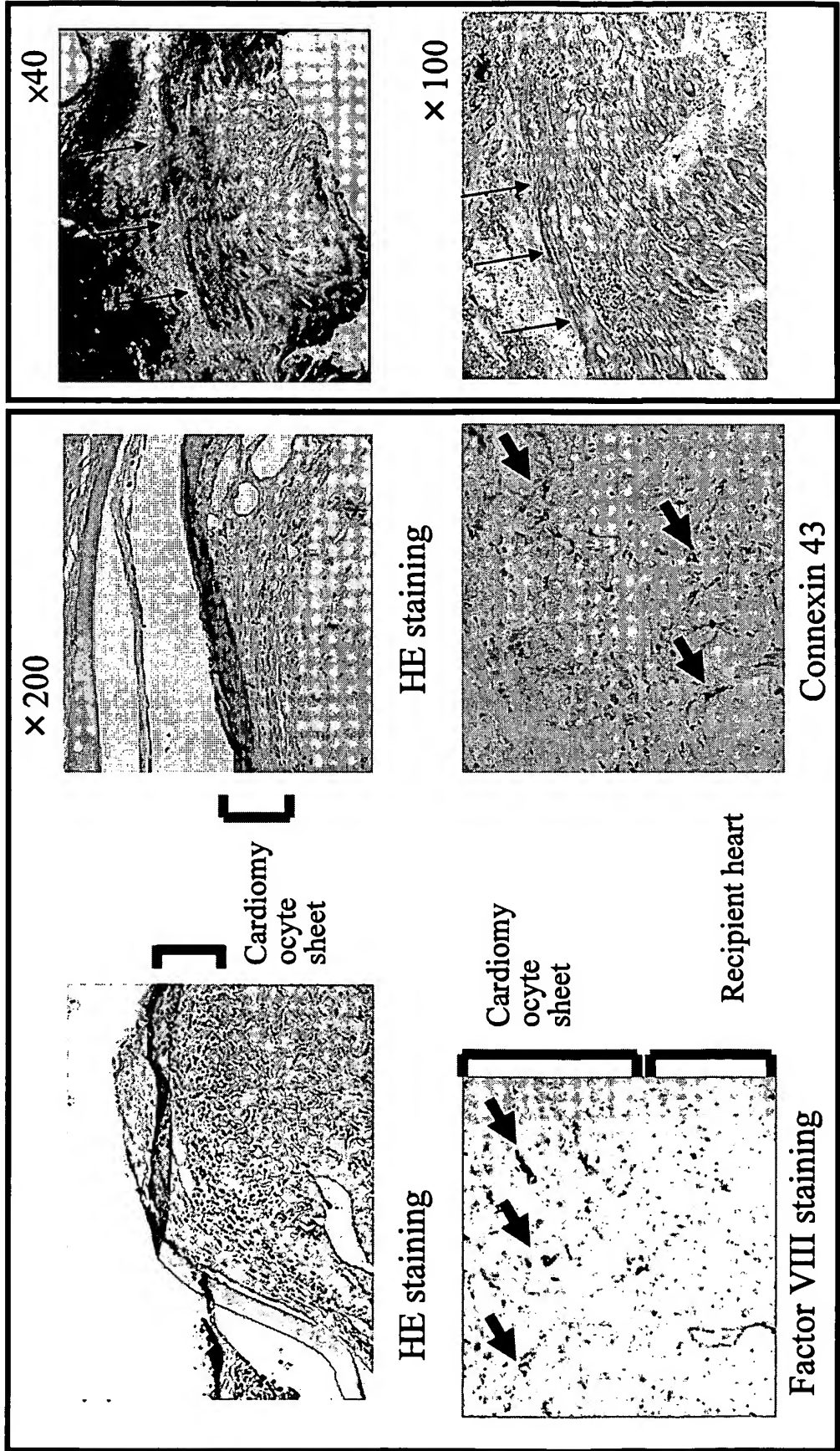
8 / 47

Tissue

FIG. 7

8 weeks after
implantation

2 weeks after implantation

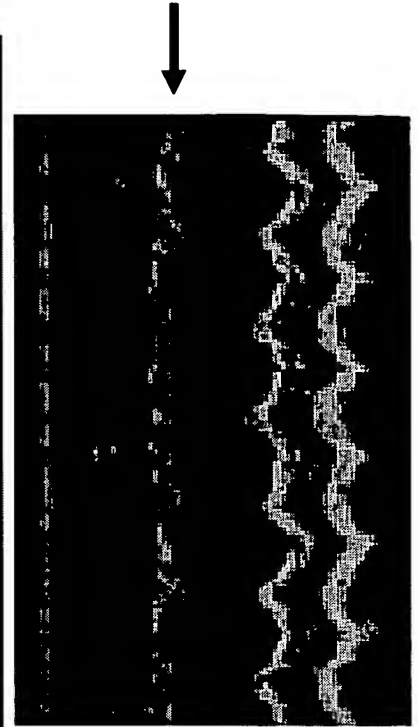
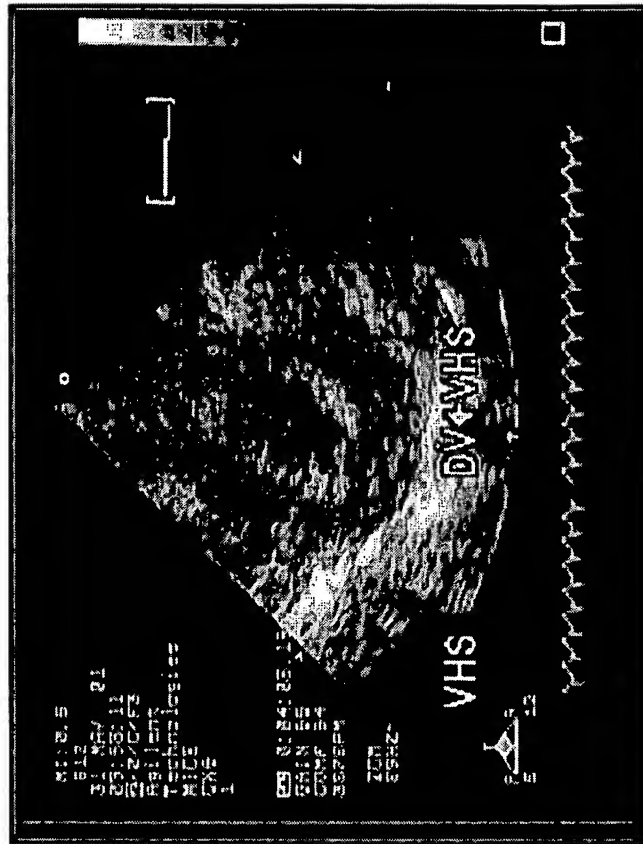
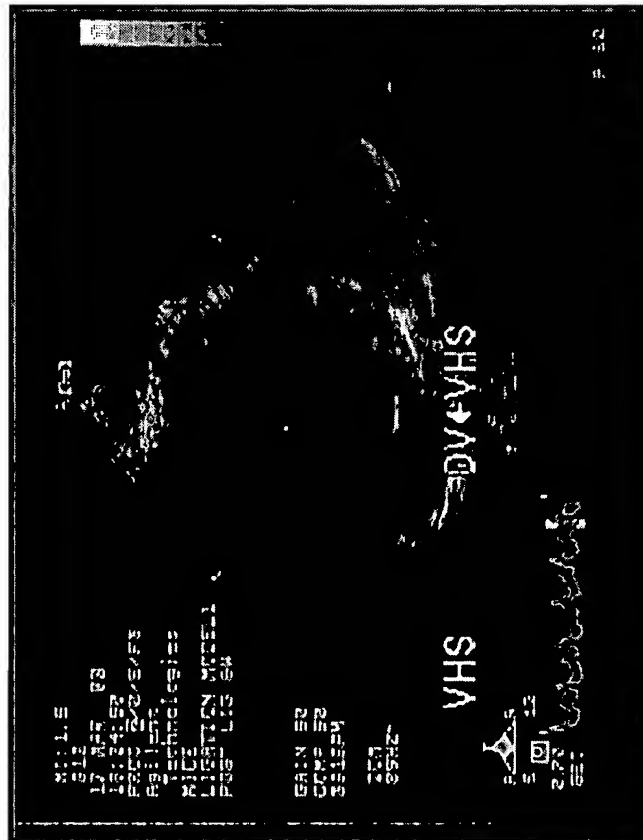


Evaluation of Cardiac function - 1

F/G.8

Control

Implantation of prosthetic tissue

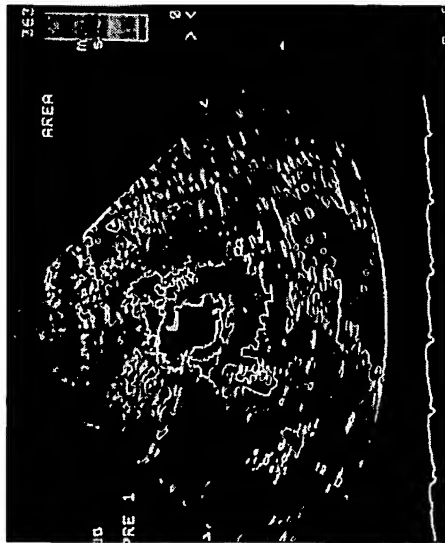


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FIG.9

Evaluation of cardiac function - 2

Base line



Implanted cardiomyocyte sheet

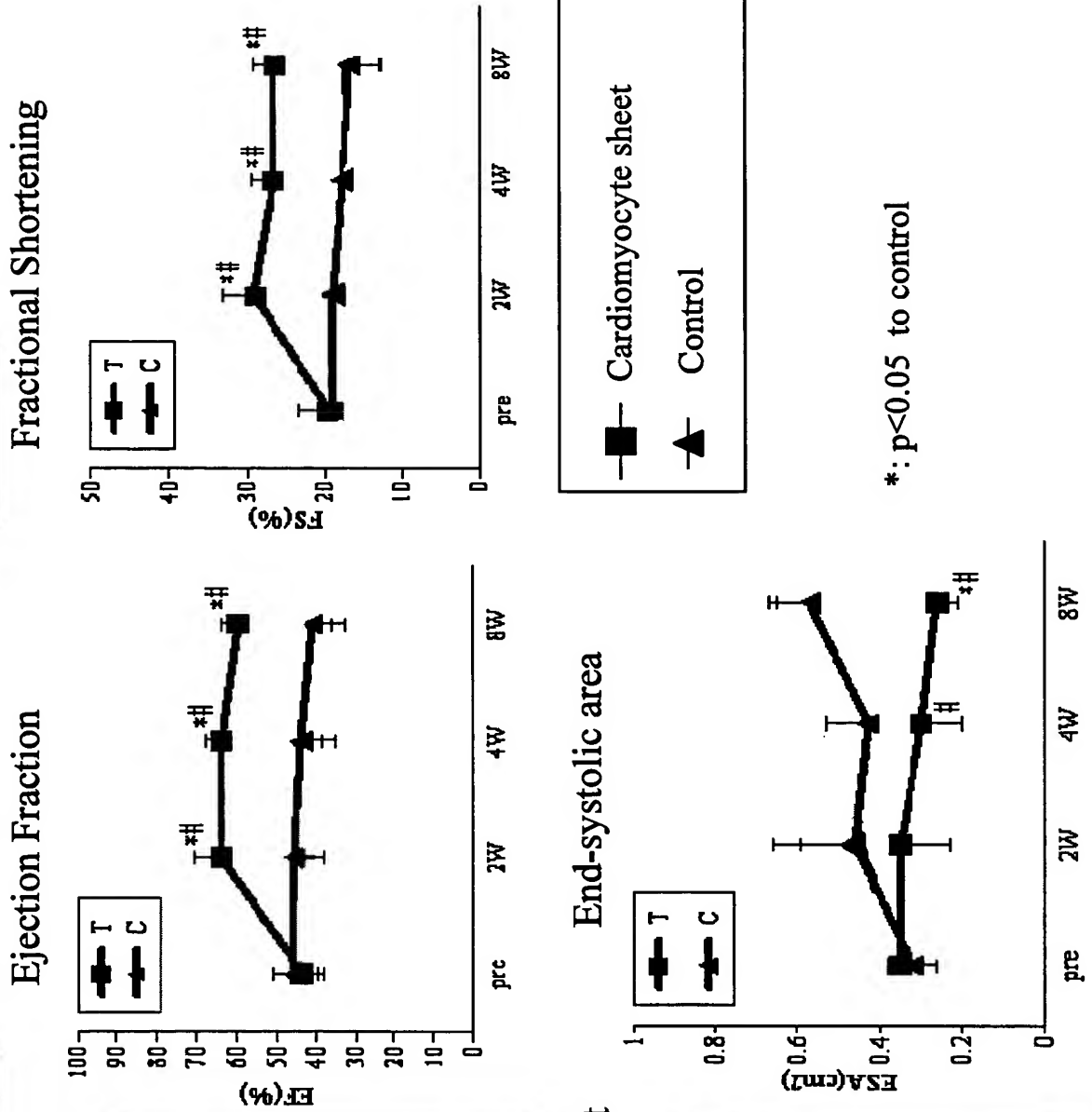
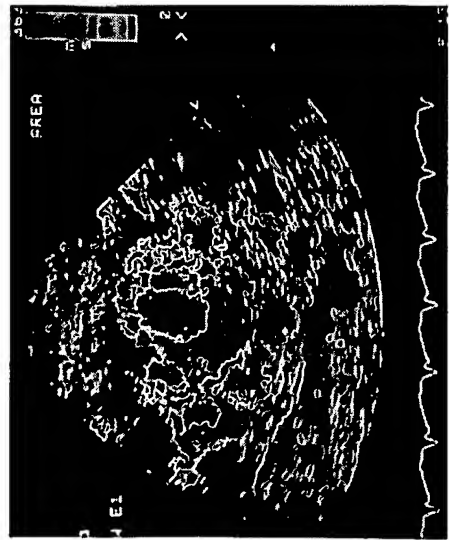
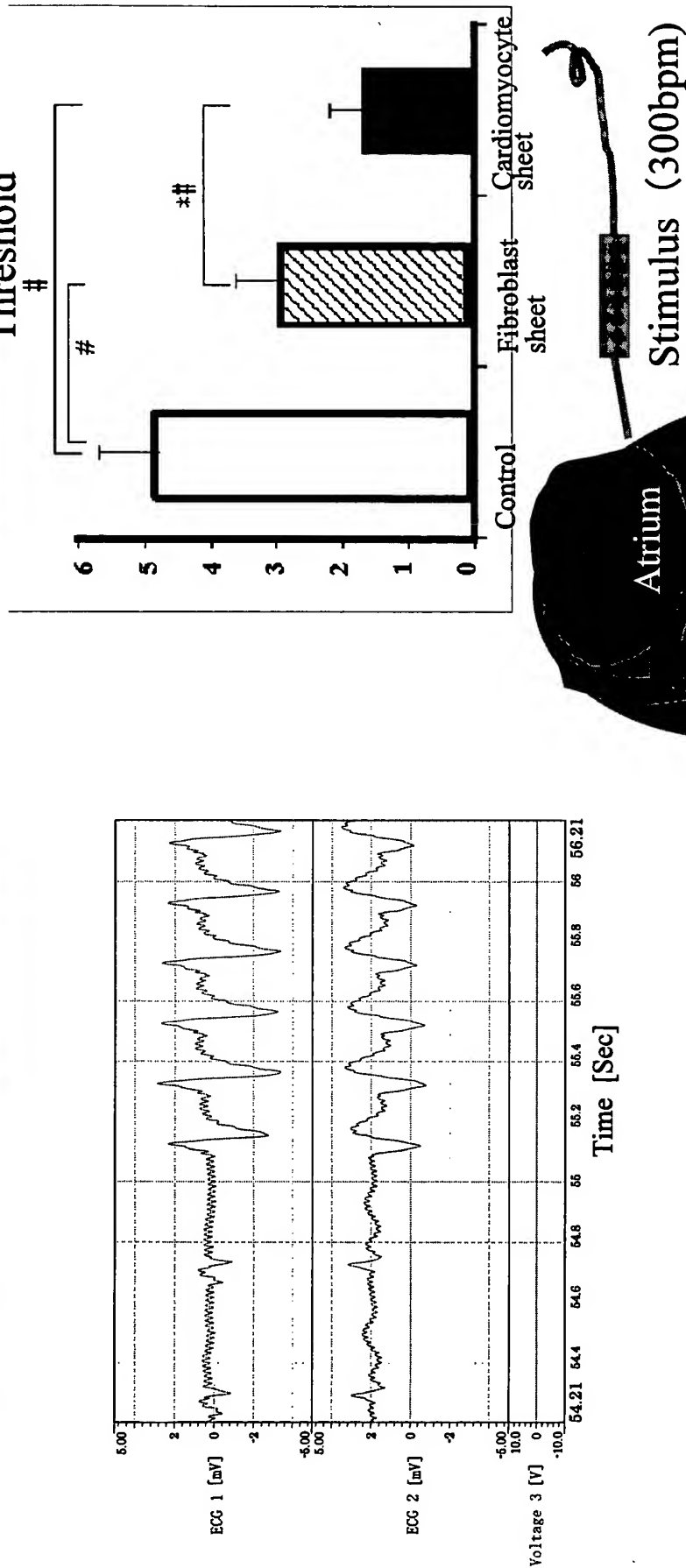


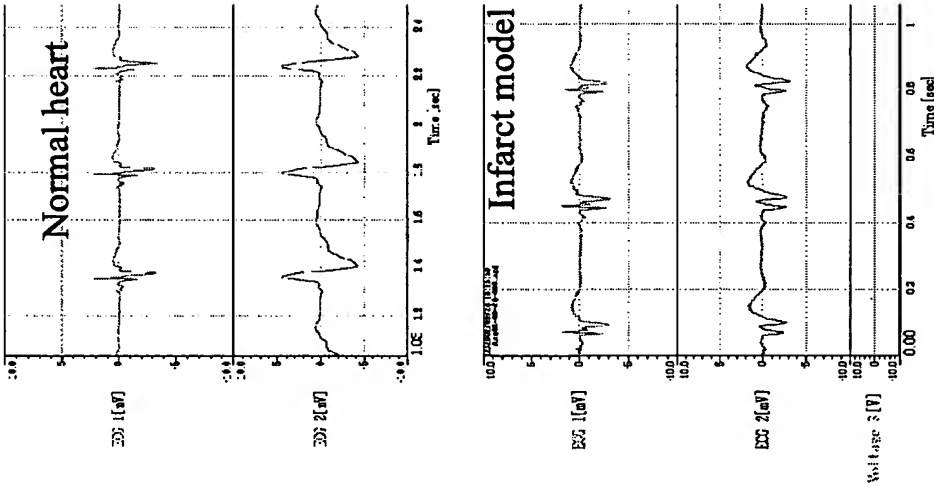
FIG.10

Electrophysiological Evaluation



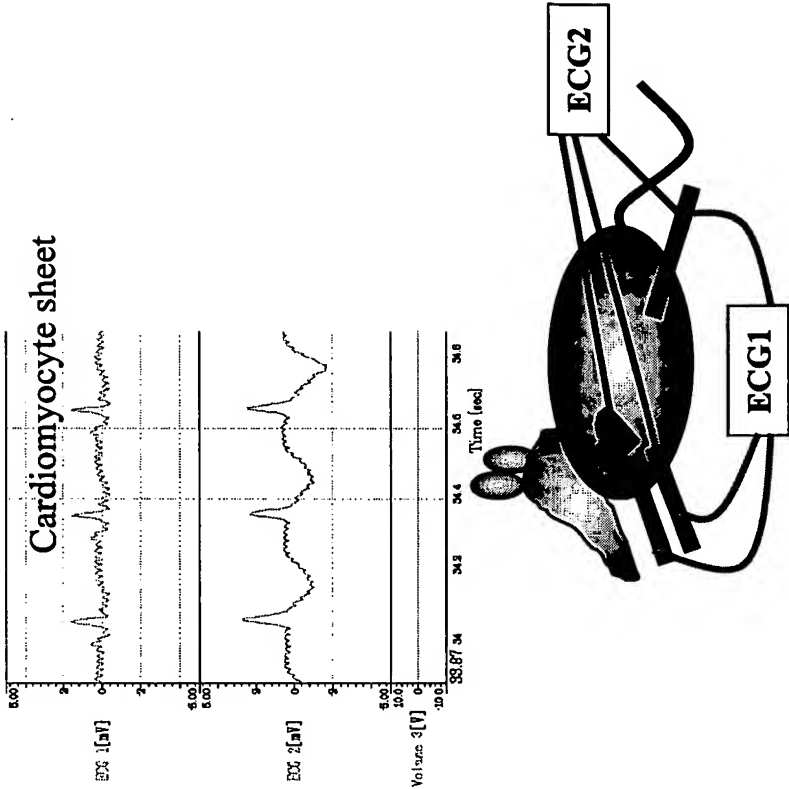
Buried electrode at
sheet implanted site

FIG.11



ECG 1:ECG (Surface)
ECG 2: Normal heart (anterior wall)
Ligation model (injured)
Prosthetic tissue implanted
(prosthetic tissue injured)

Electrophysiological Evaluation



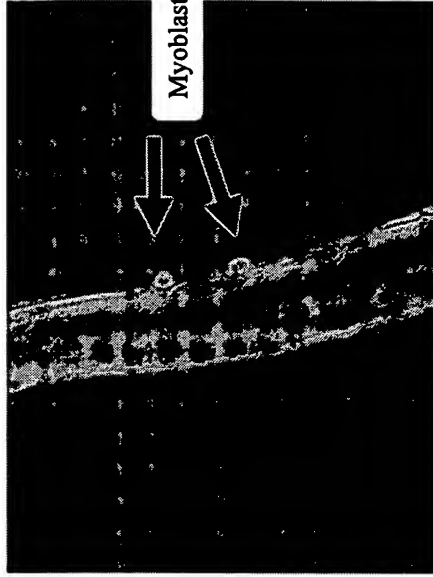
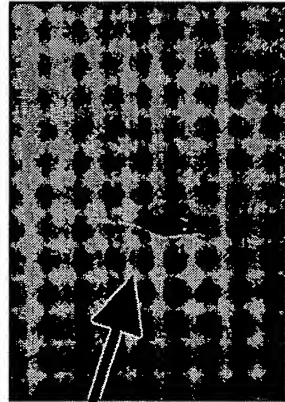
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FIG.12

Isolation and culture of myoblast



Harvested Muscle



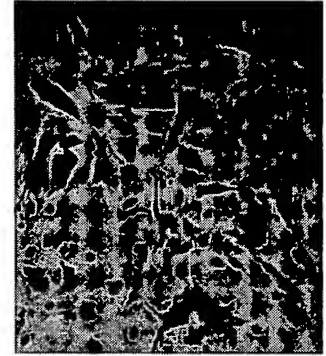
Collagenase 37°C, 30min



Myoblasts in culture

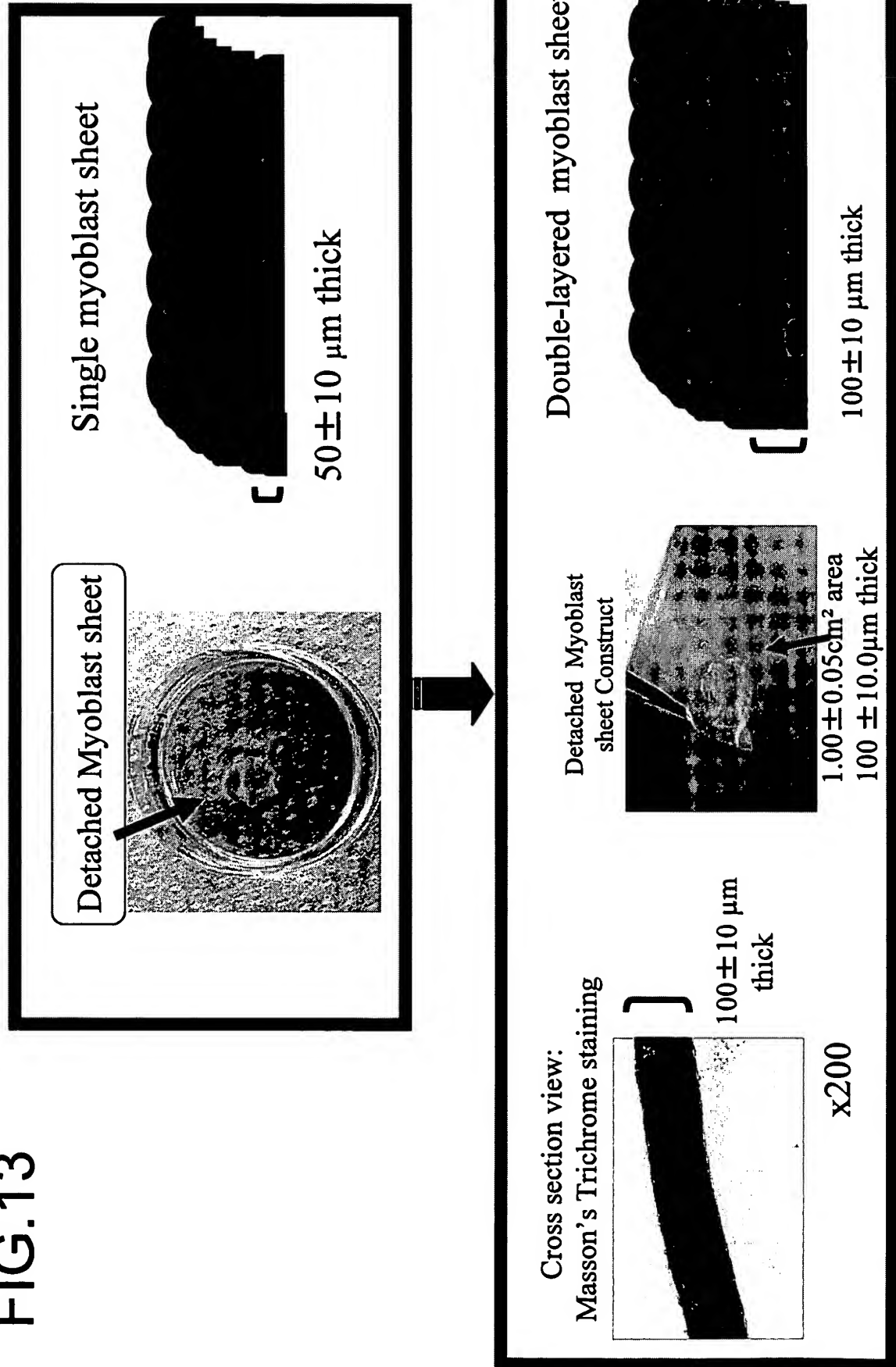


Desmin stained Myoblasts



Methods: Myoblast Sheet Construction

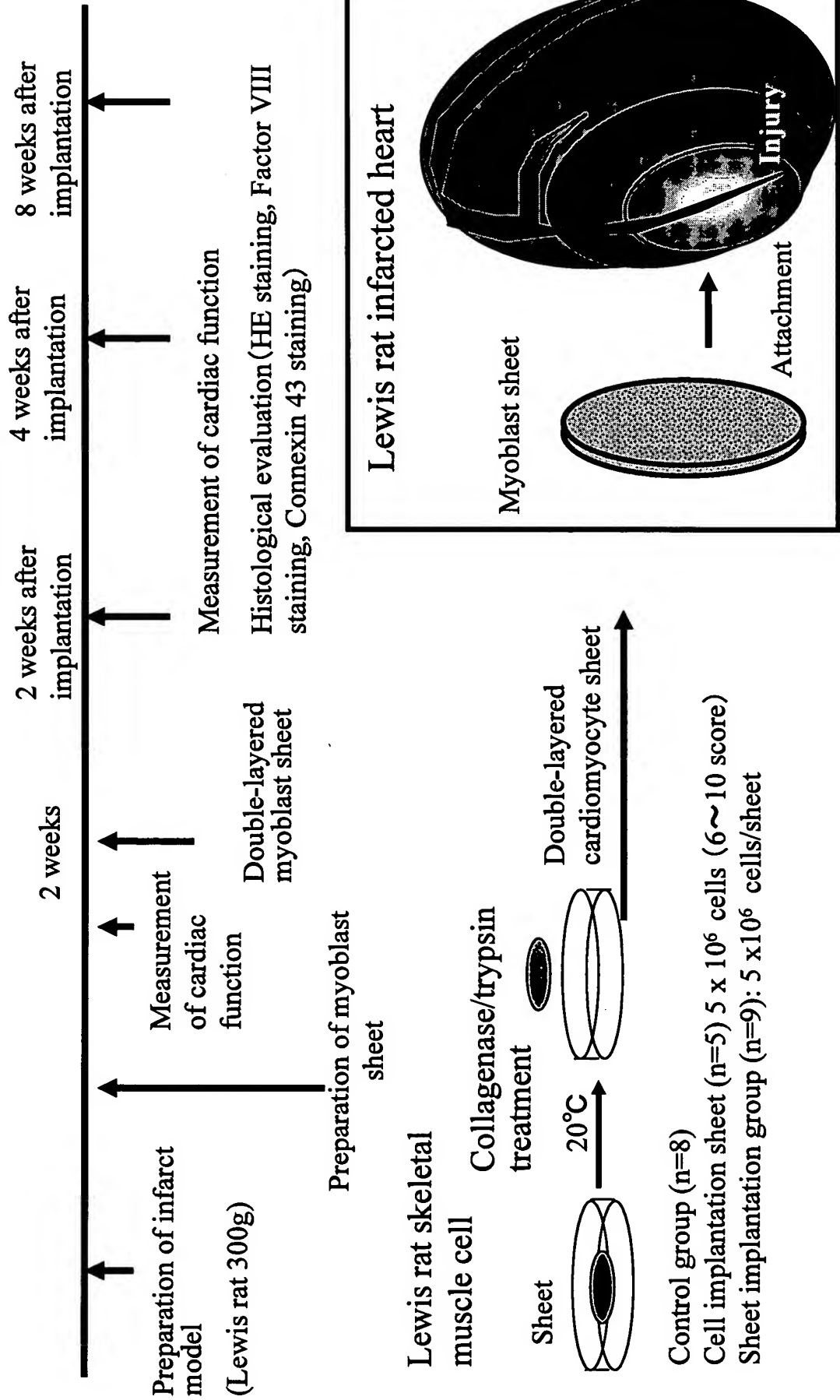
FIG.13



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FIG.14

Experimental Protocol



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Myoblast sheet: 4W post implantation

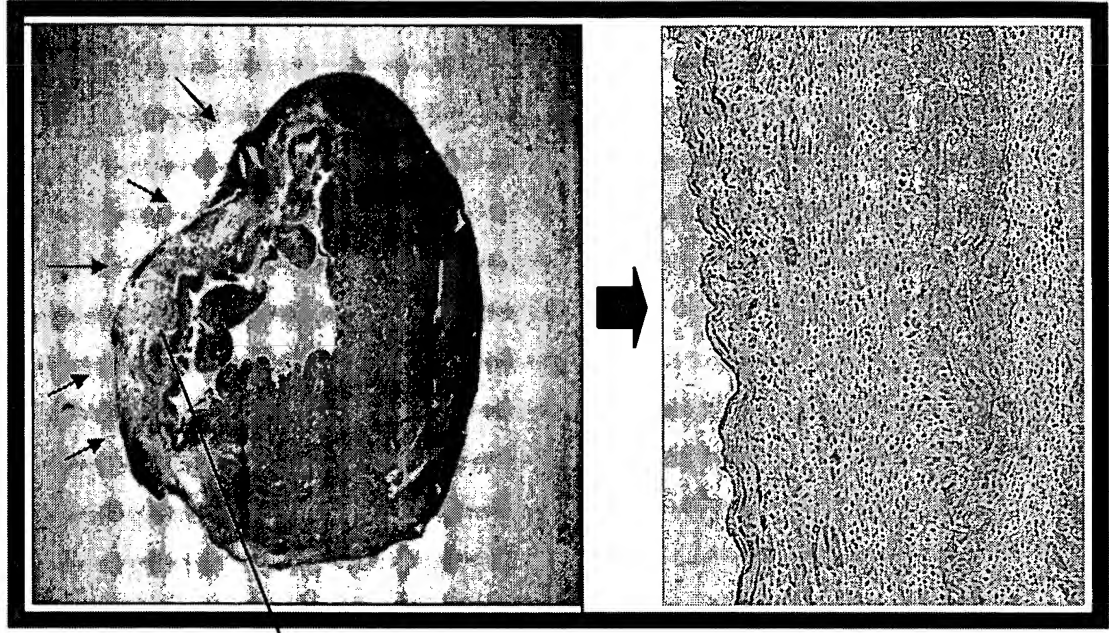
FIG.15

Implanted myoblasts



x1000

HE staining



x10

x200

Myoblast sheet Implantation procedure

FIG.16

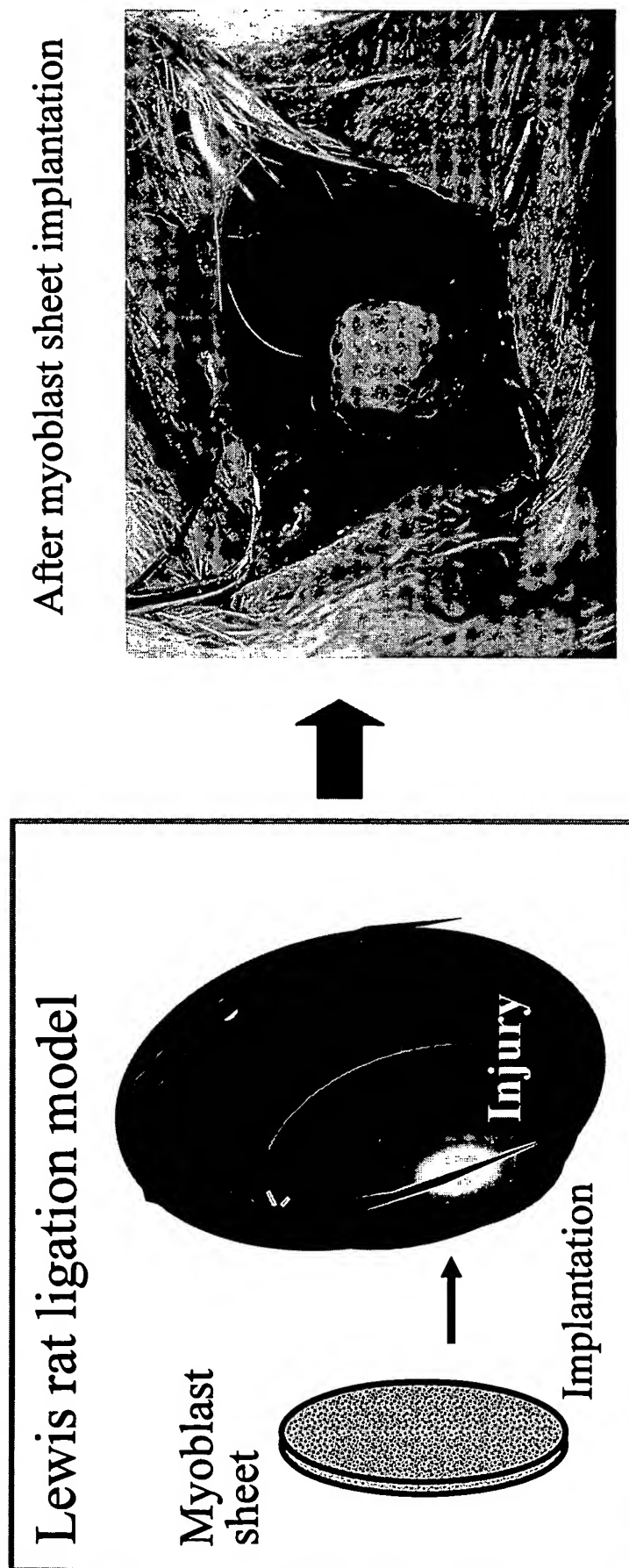


FIG.17 HistologyMasson's Trichrome staining

Myoblast prosthetic tissue group
4W post



x10

Myoblast injection group
4W post

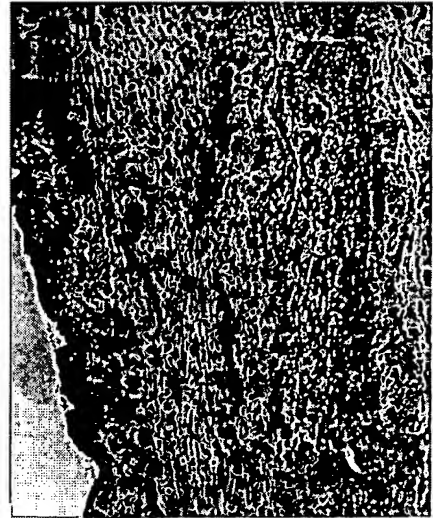


x10

Control group
4W post



x10



x200

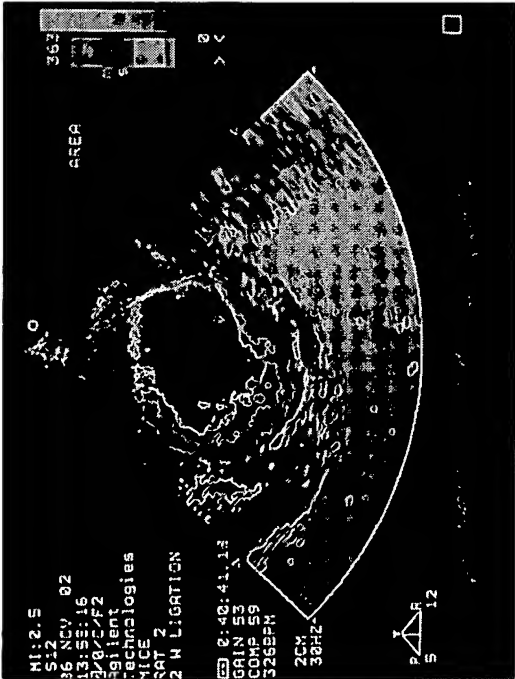
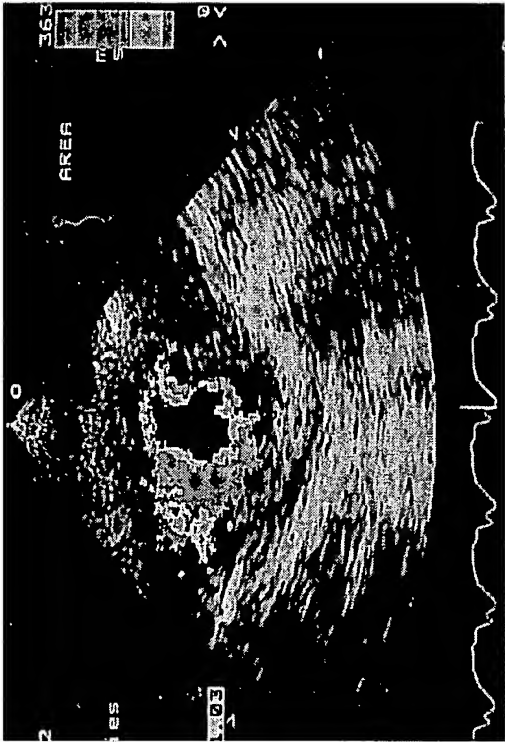


x40



x40

FIG.18 CKA



M-mode analysis

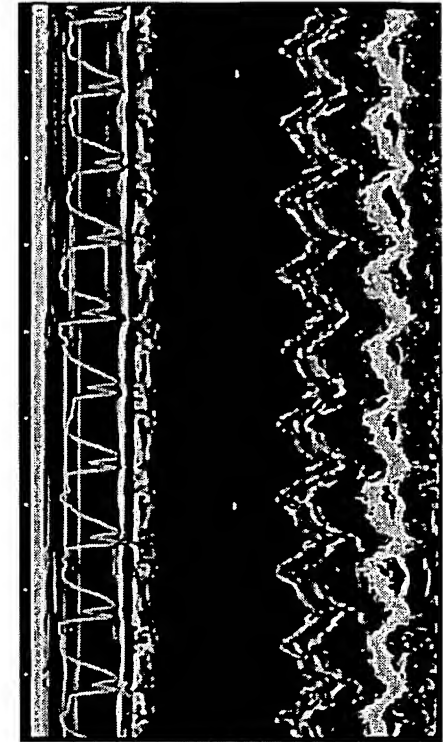
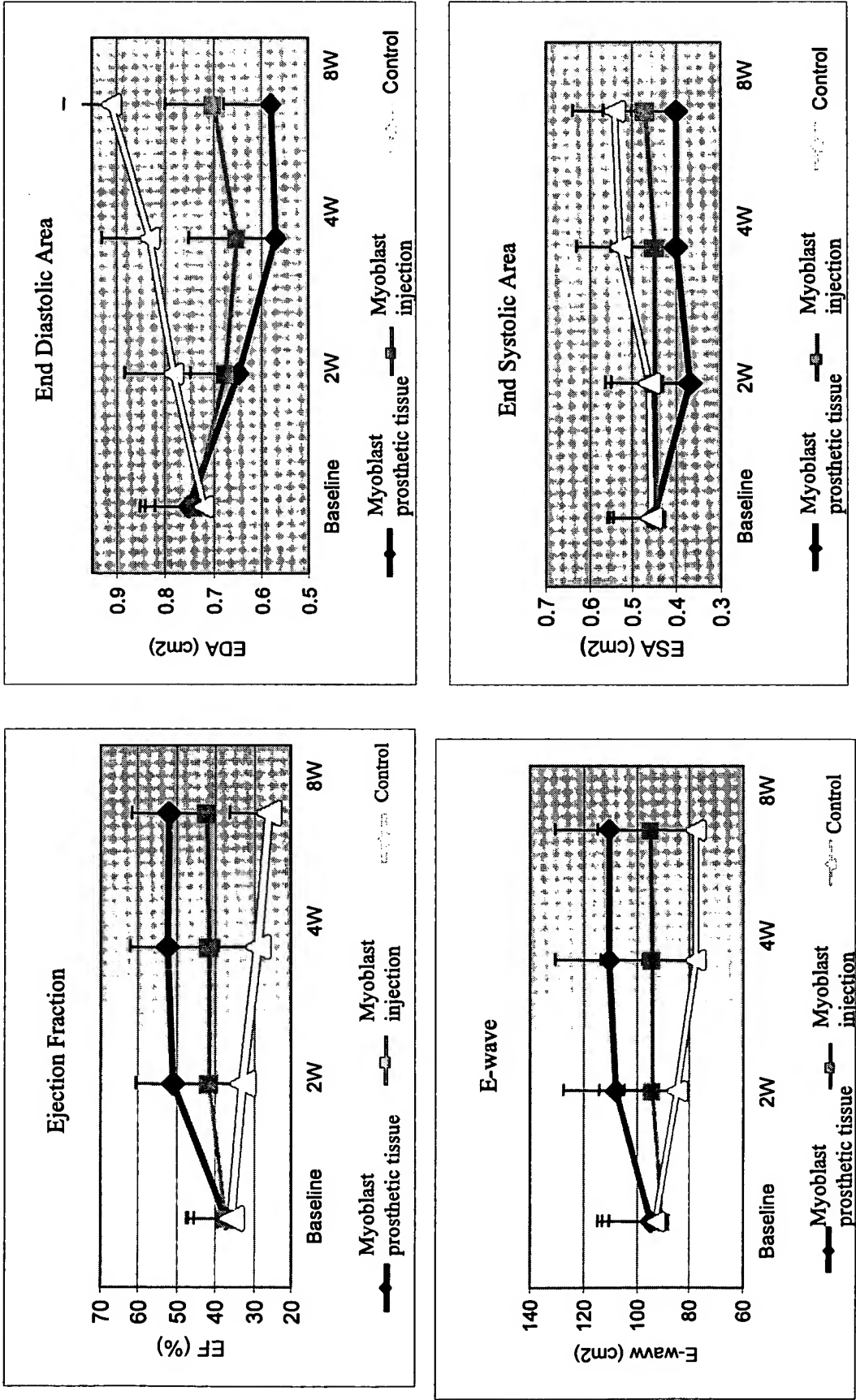


FIG.19



#P< 0.05 for control; *P< 0.05 to for injection needle group

FIG.20

Anterior Wall Thickness Comparison

Myoblast prosthetic tissue



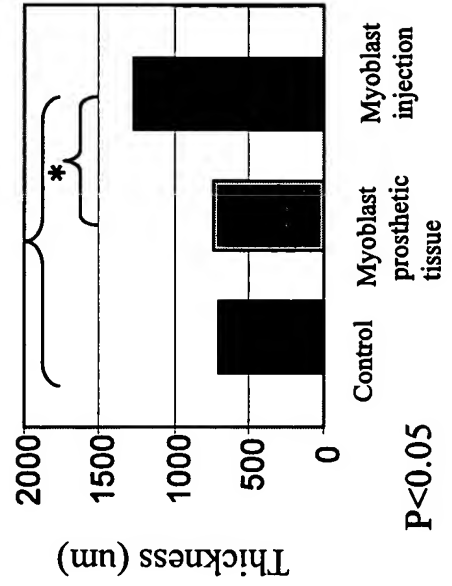
x40

Myoblast injection



x40

Anterior wall thickness



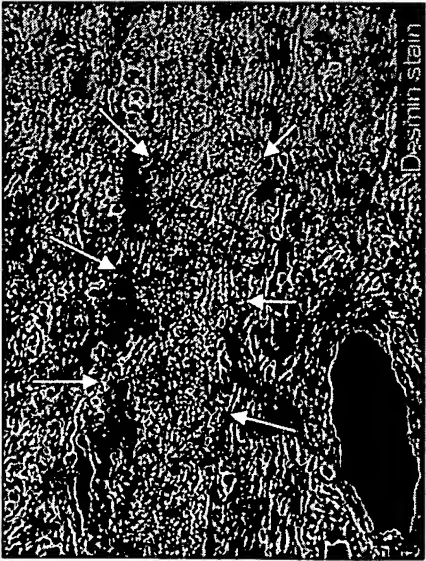
Control



x40

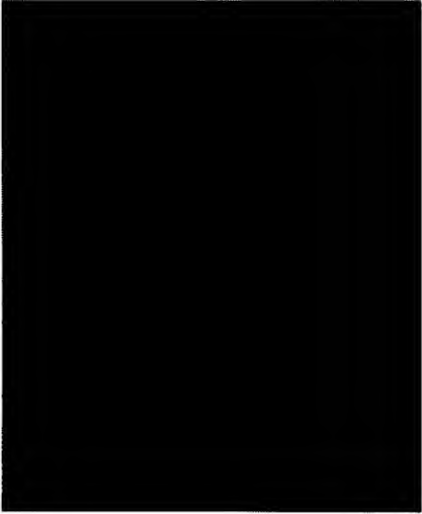
FIG.21

Myoblast sheet:
Desmin Staining



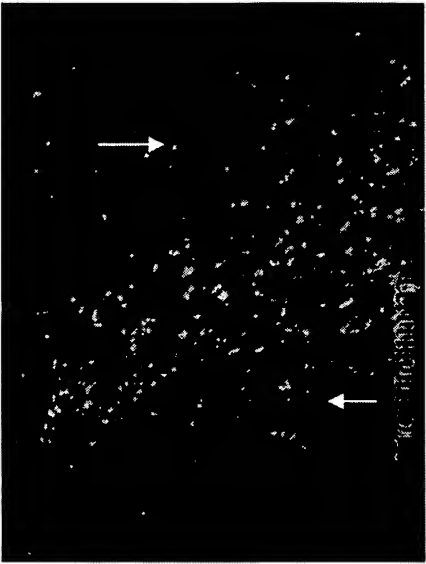
x100

Control group (GFP)



x100

Myoblast prosthetic
tissue group (GFP)



x100

22 /47

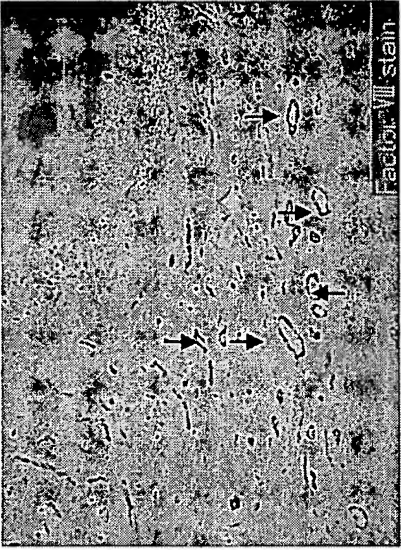
Factor VIII staining

Myoblast injection



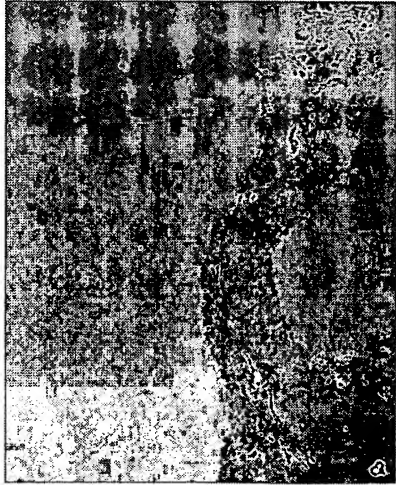
x40

Myoblast prosthetic tissue



x40

Control



x40

FIG.22A

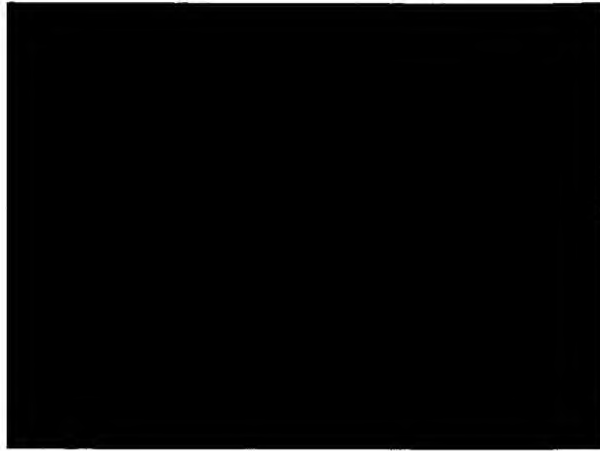


FIG.22B



FIG.22C



FIG.22D

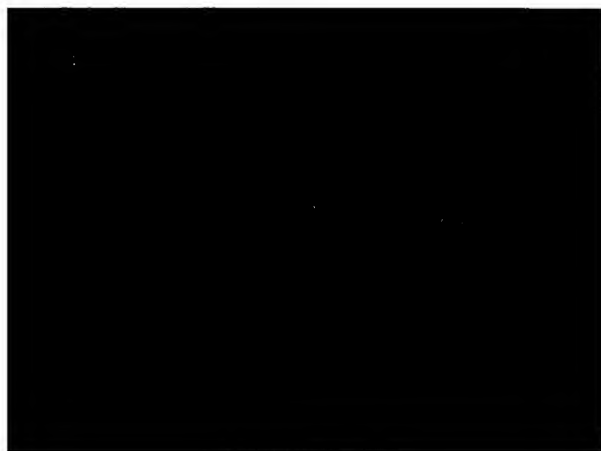


FIG.22E



FIG.22F

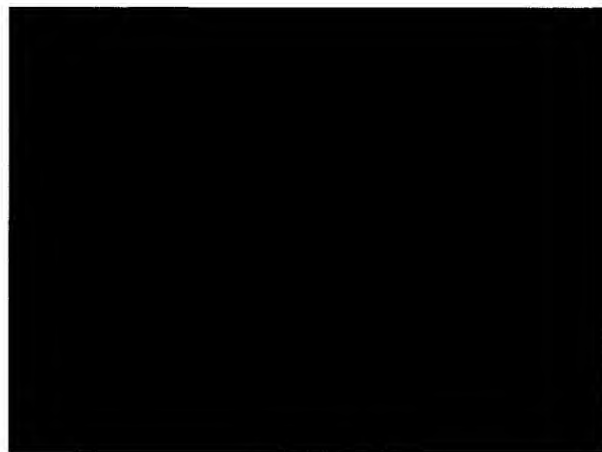


FIG.23A



FIG.23B

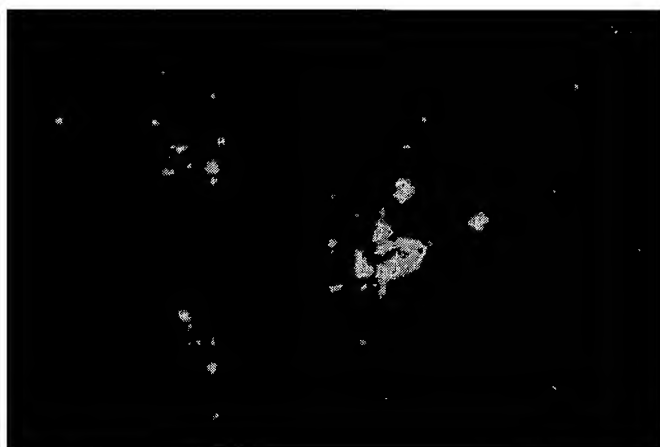


FIG.23C



FIG.24A

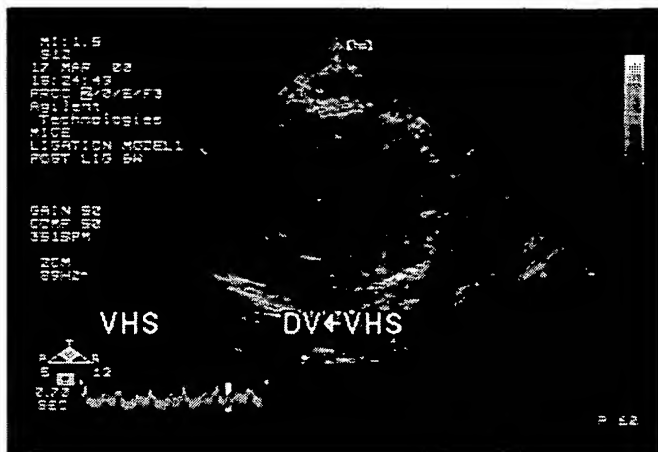


FIG.24B

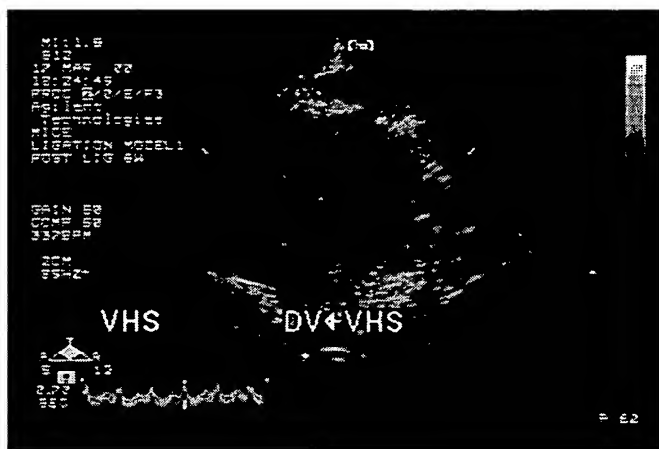


FIG.24C

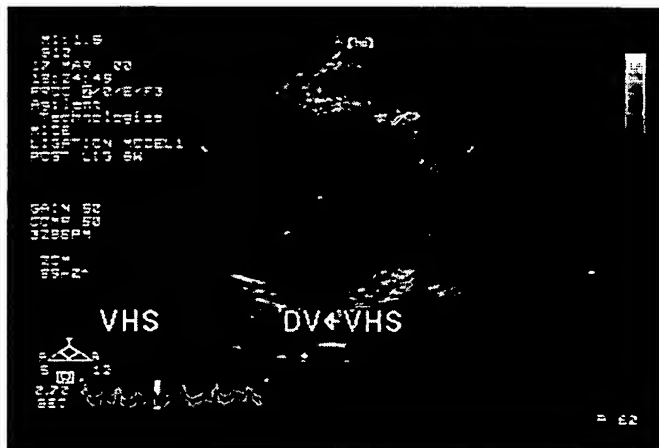


FIG.25A

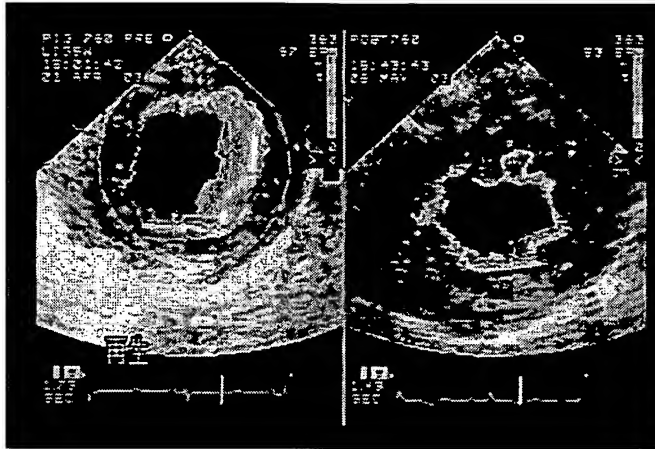


FIG.25B

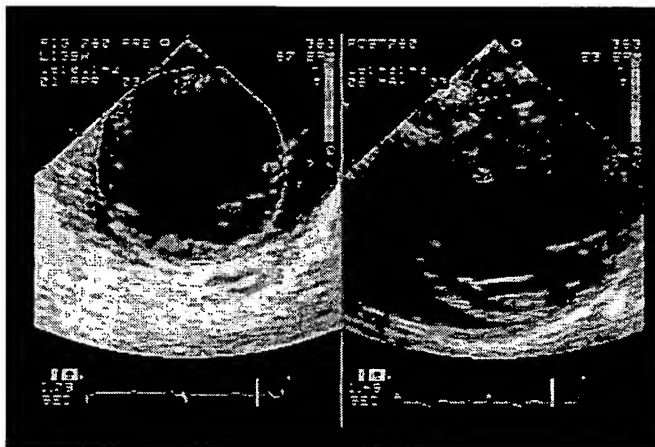


FIG.25C

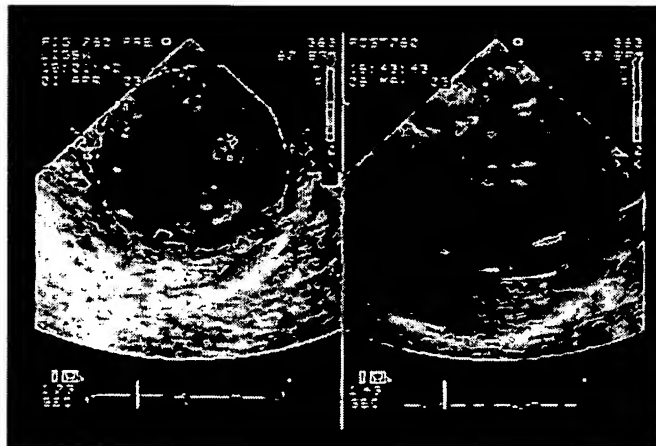


FIG.26A

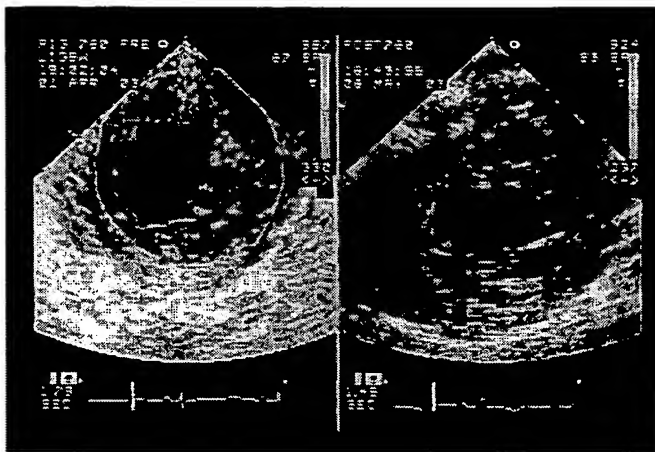


FIG.26B

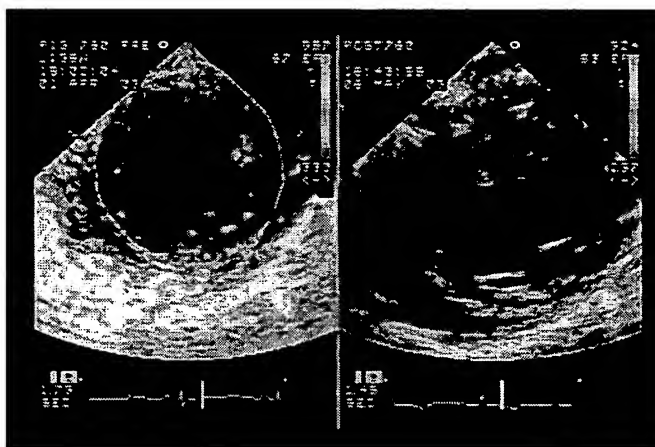


FIG.26C

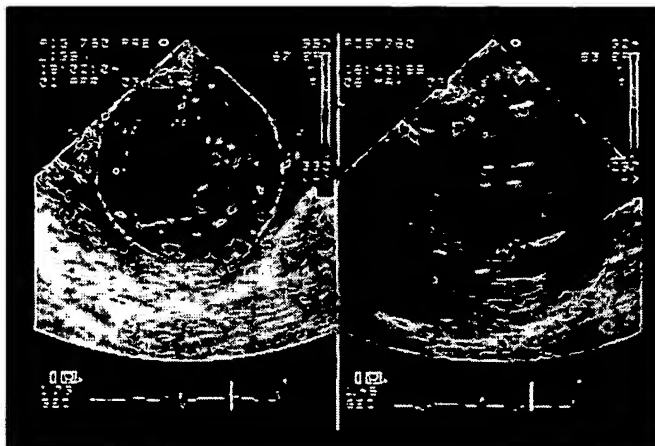


FIG.28

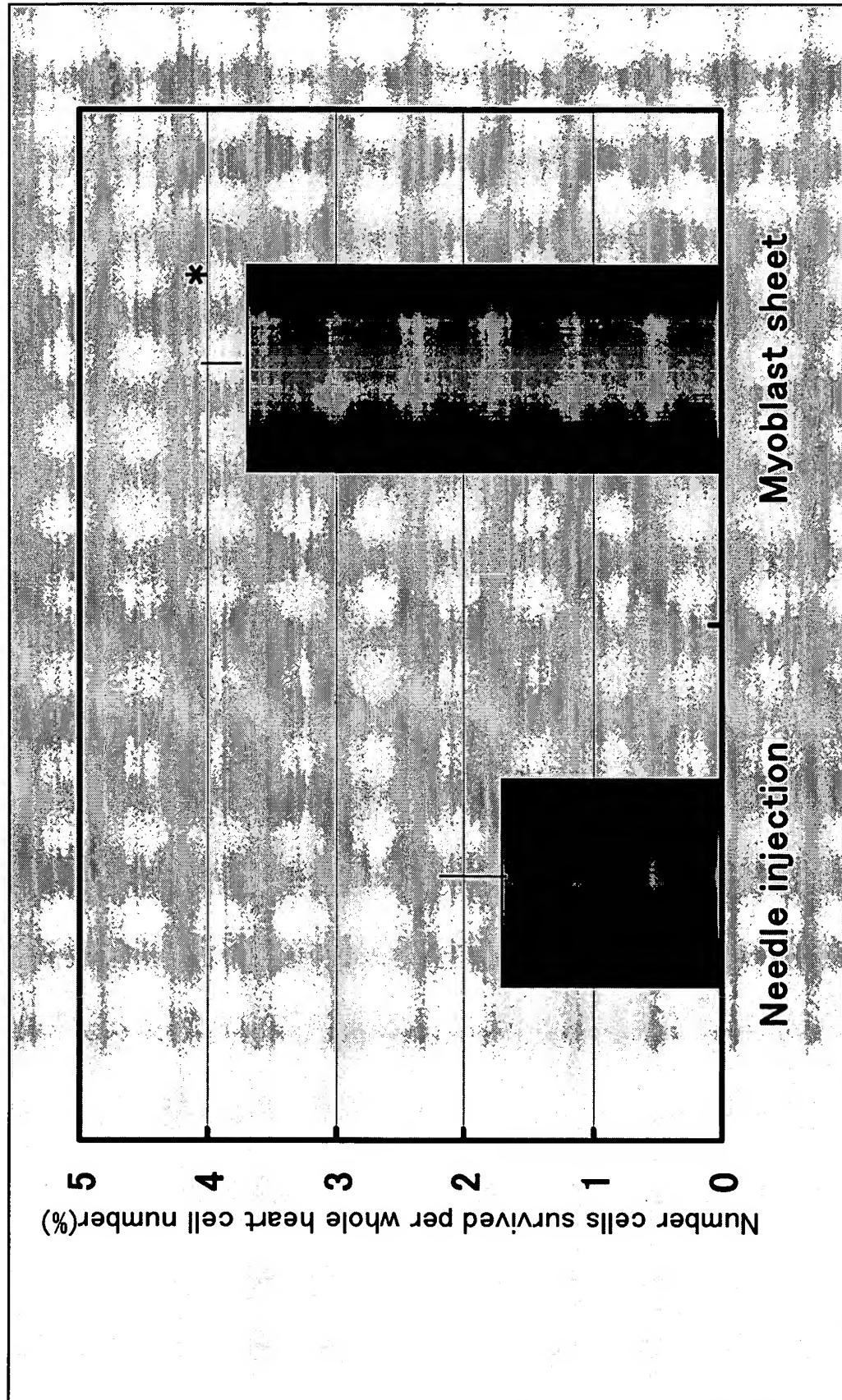
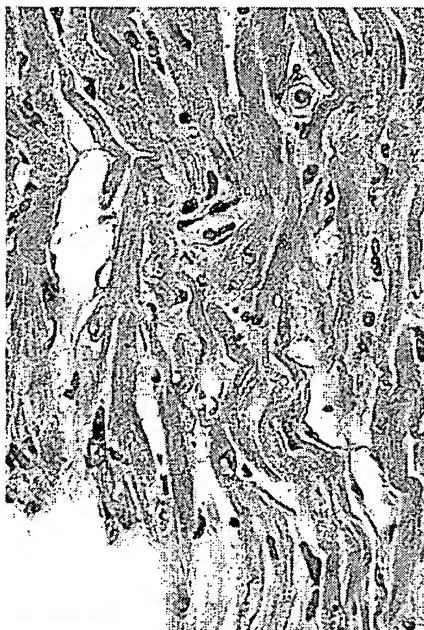


FIG.29

Masson's Trichrome staining x400



HE staining x400



MHC fast x400



MHC slow x400

FIG.30A Tissue (Masson's Trichrome staining)

Myoblast sheet
4W post implantation

x10



Myoblast implantation
(needle injection)

4W post implantation x10



Control

x10



x40

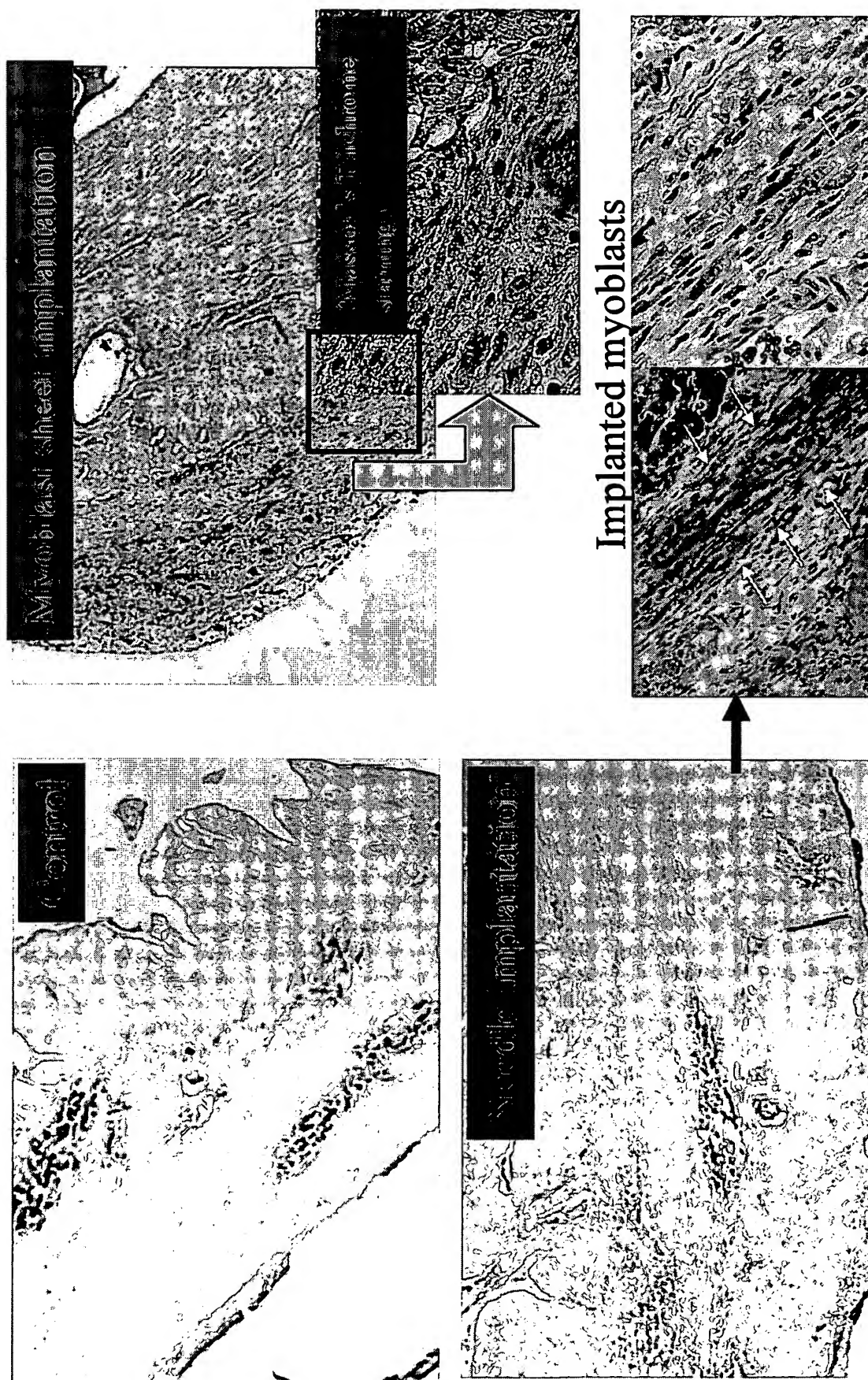


x40



x40

FIG.30B



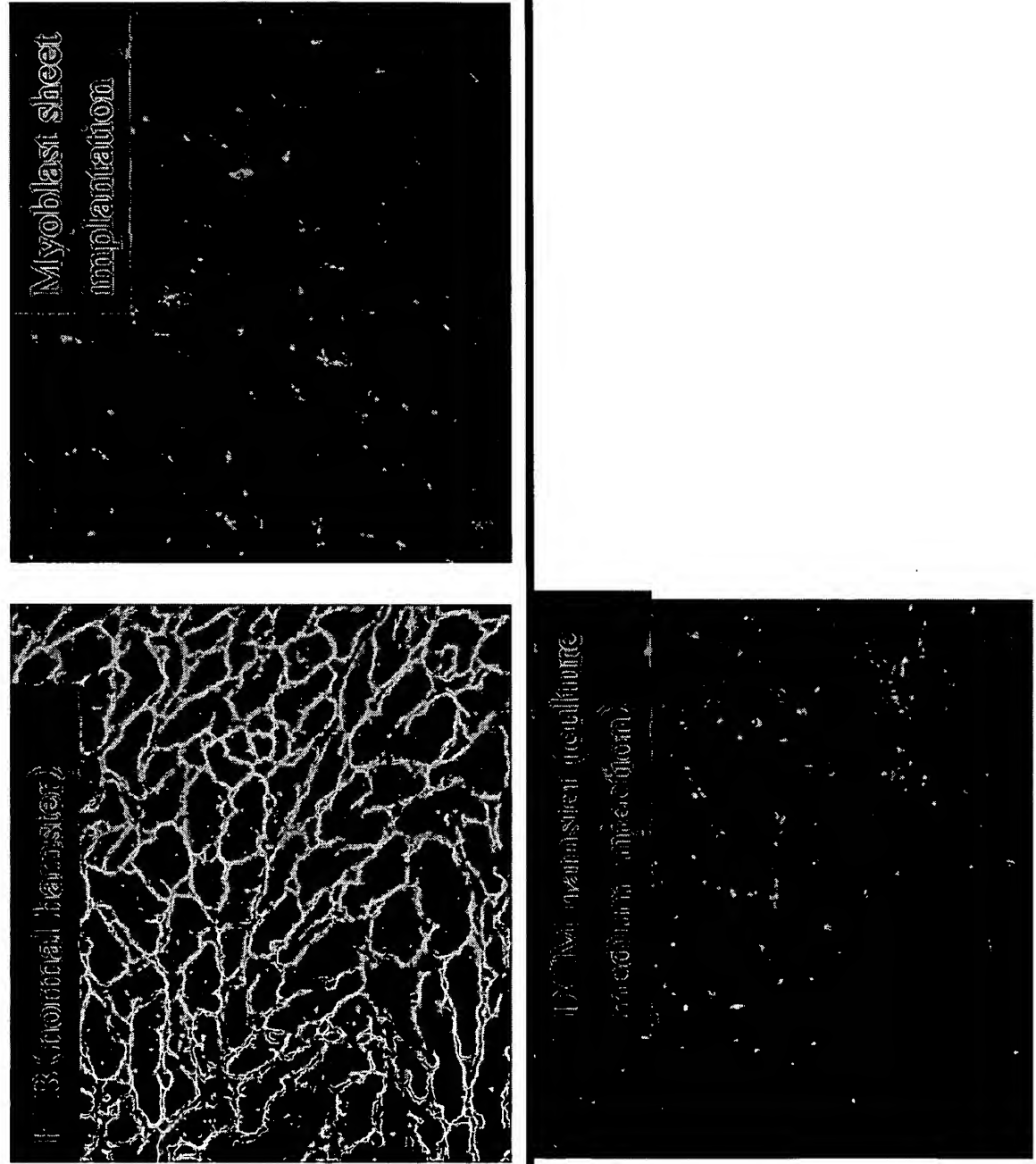


FIG.30C

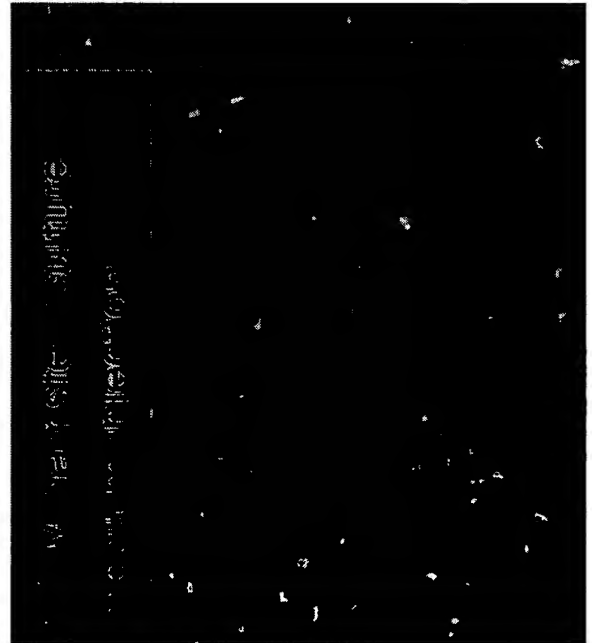
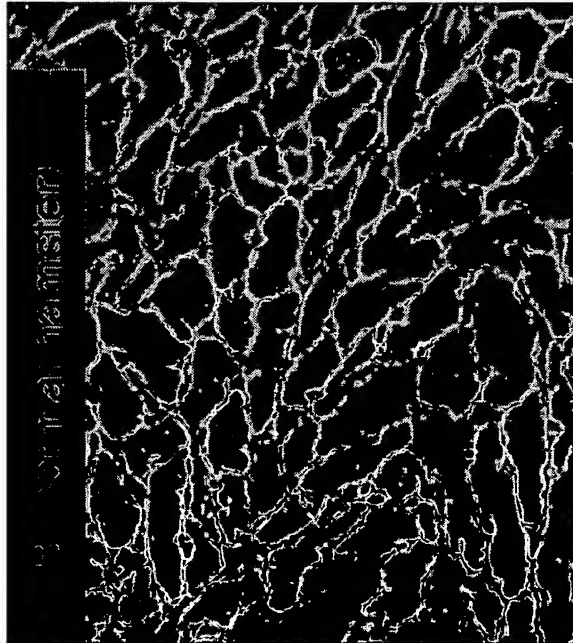


FIG.30D

FIG.31 Survival rate of implanted cell

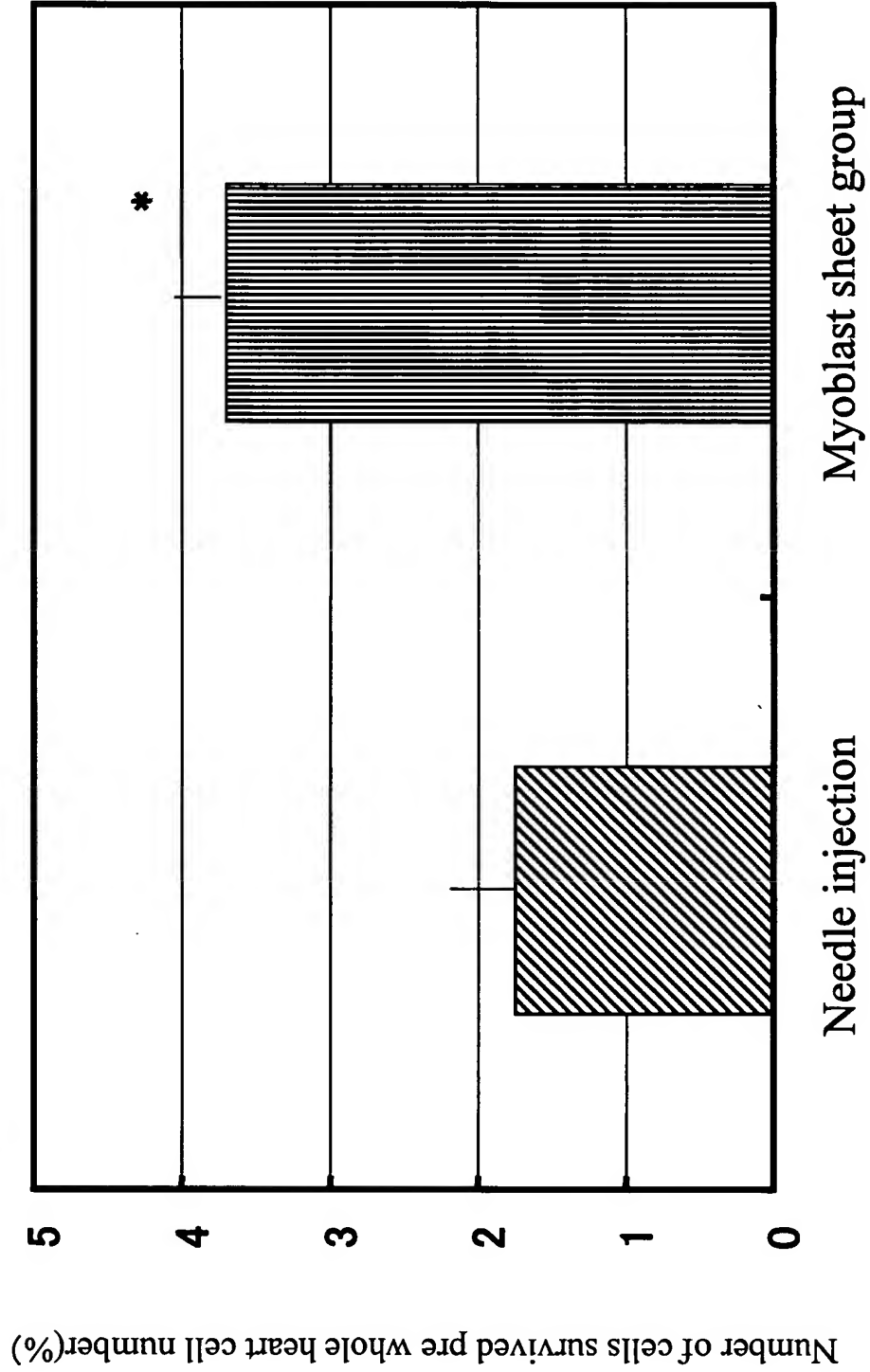
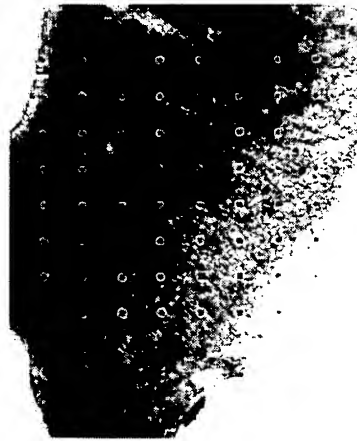


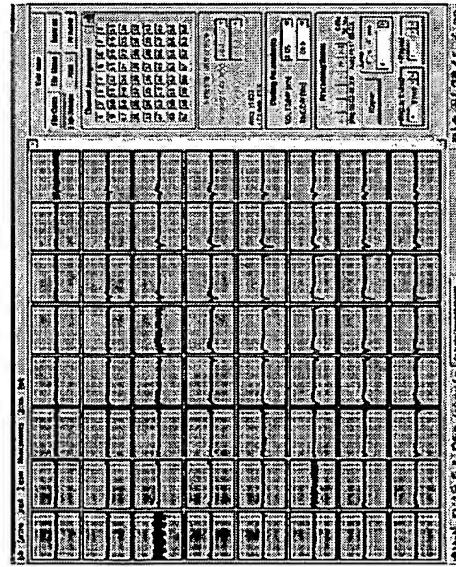
FIG.32

Electrical properties of myoblast sheet

MED system



Cardiomyocyte sheet



Myoblast sheet

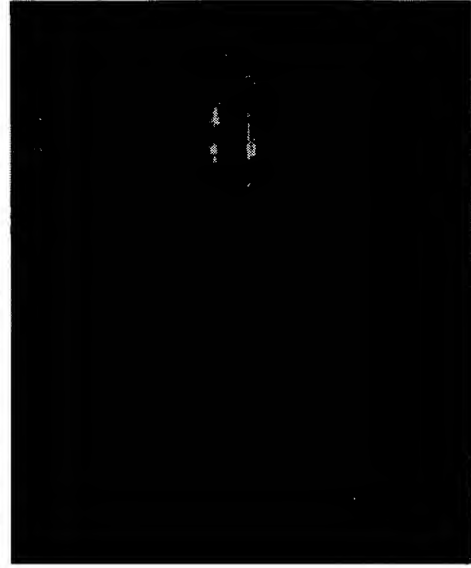
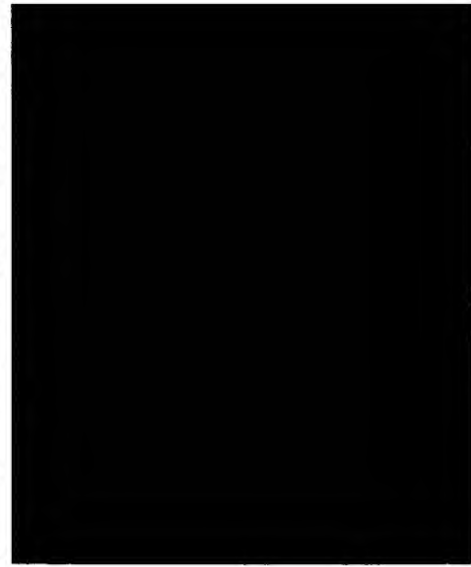
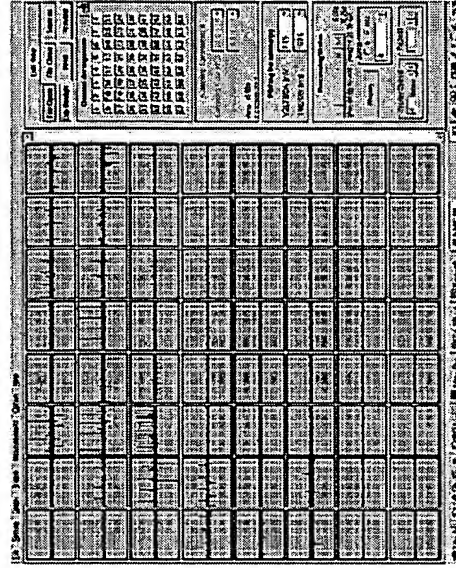
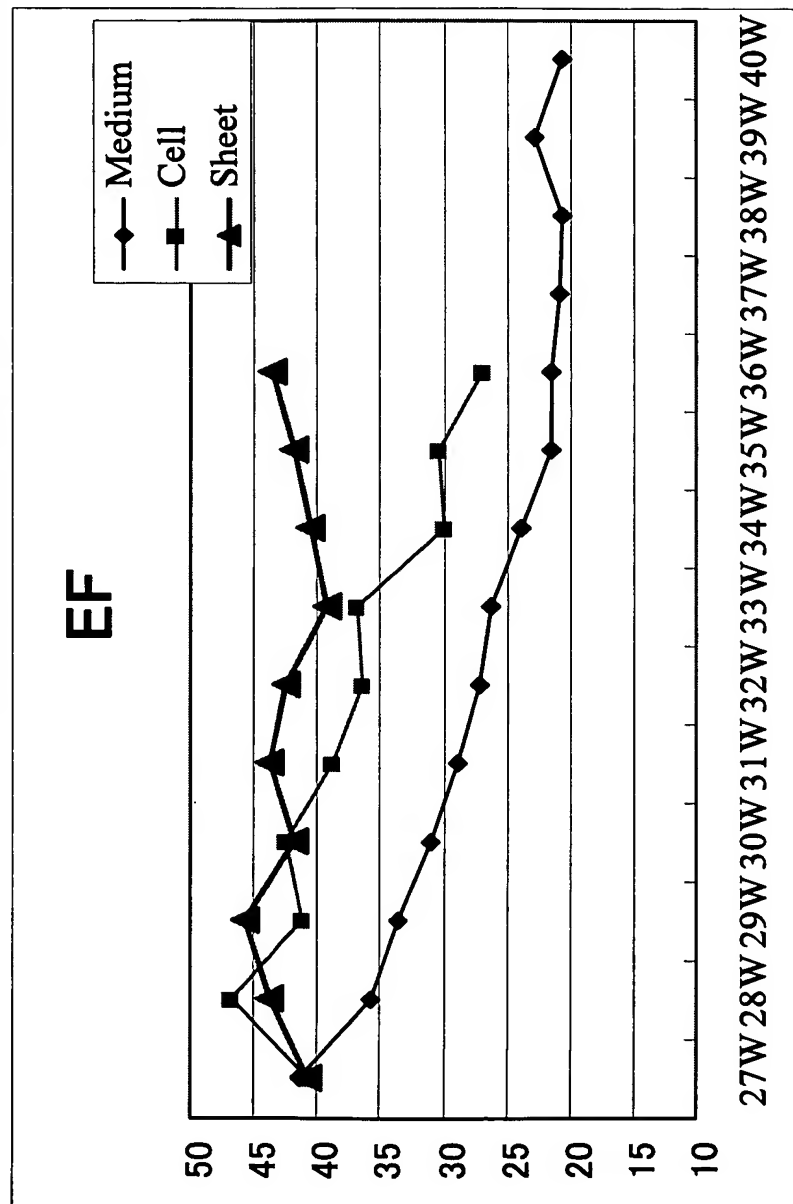


FIG.33A Myoblast sheet implantation to dilated cardiomyopathic hamster



HE staining



Masson's Trichrome staining

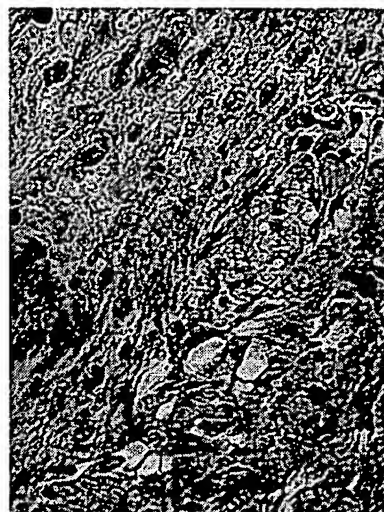
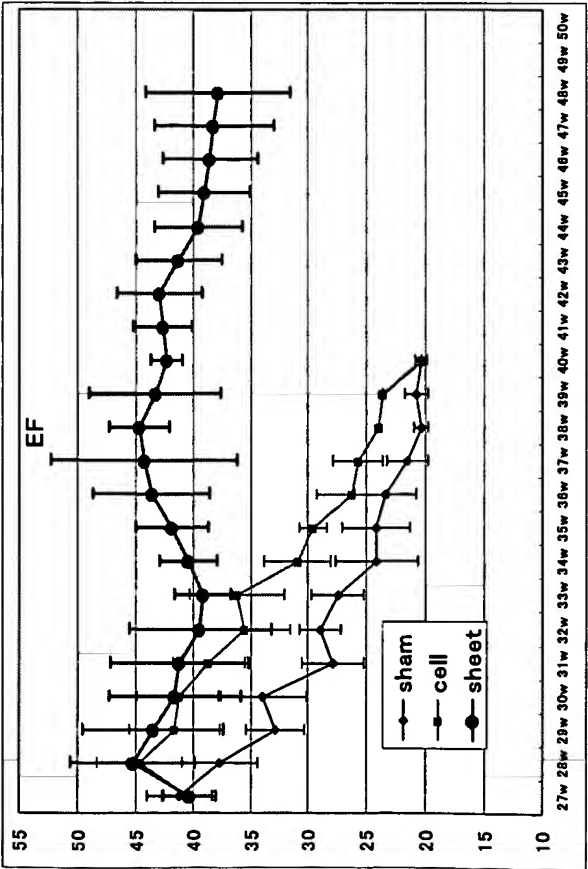


FIG.33B

Left ventricular end-systolic diameter



Left ventricular end-diastolic diameter

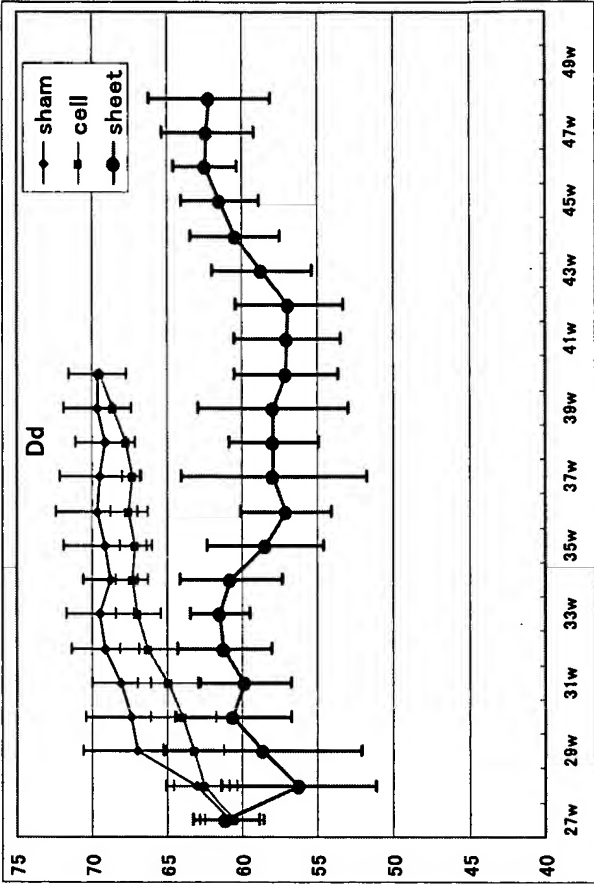
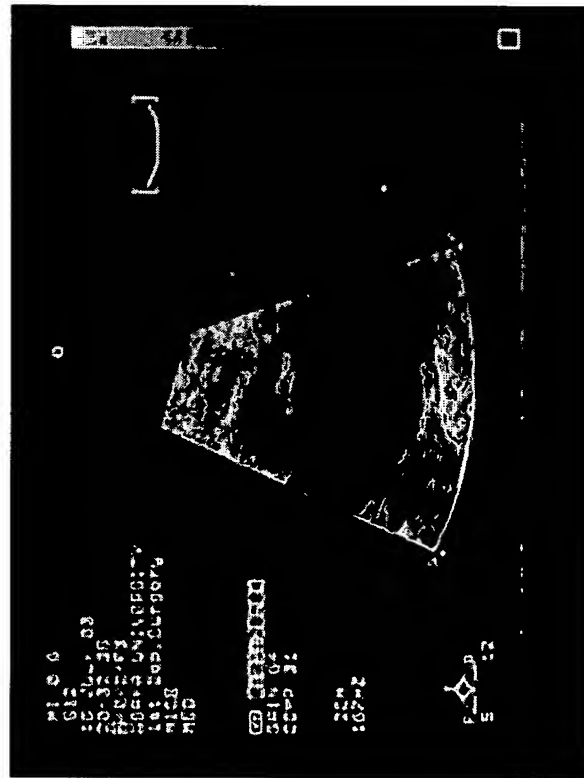


FIG. 33C

Control group



Myoblast sheet implantation group

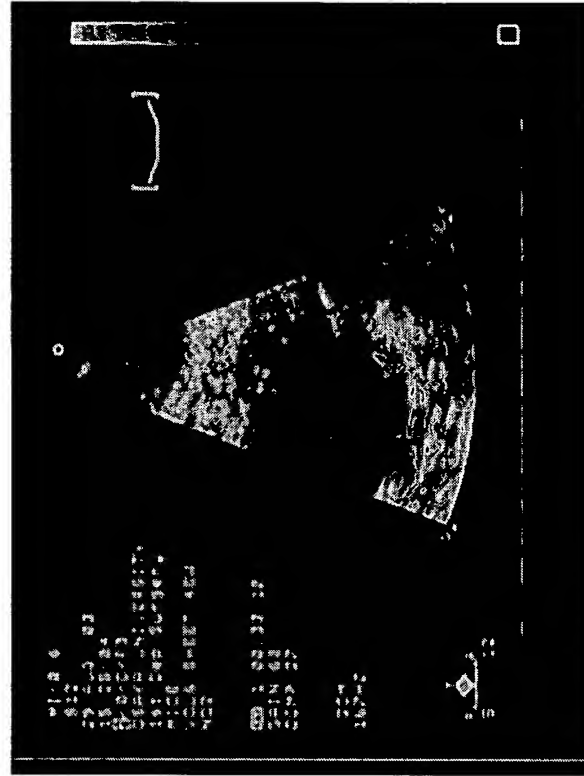


FIG.34 Myoblast sheet implantation into pig infarction model

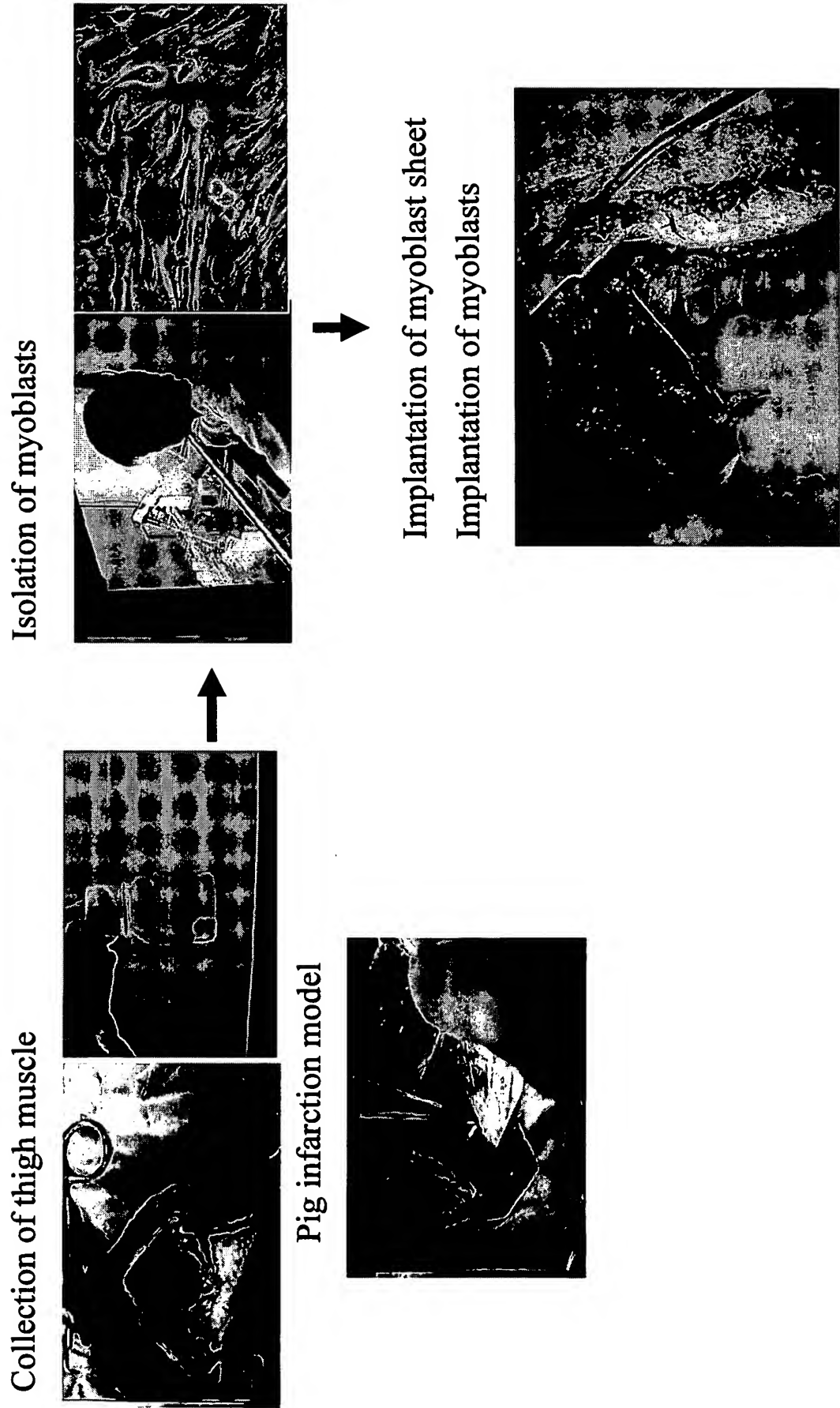
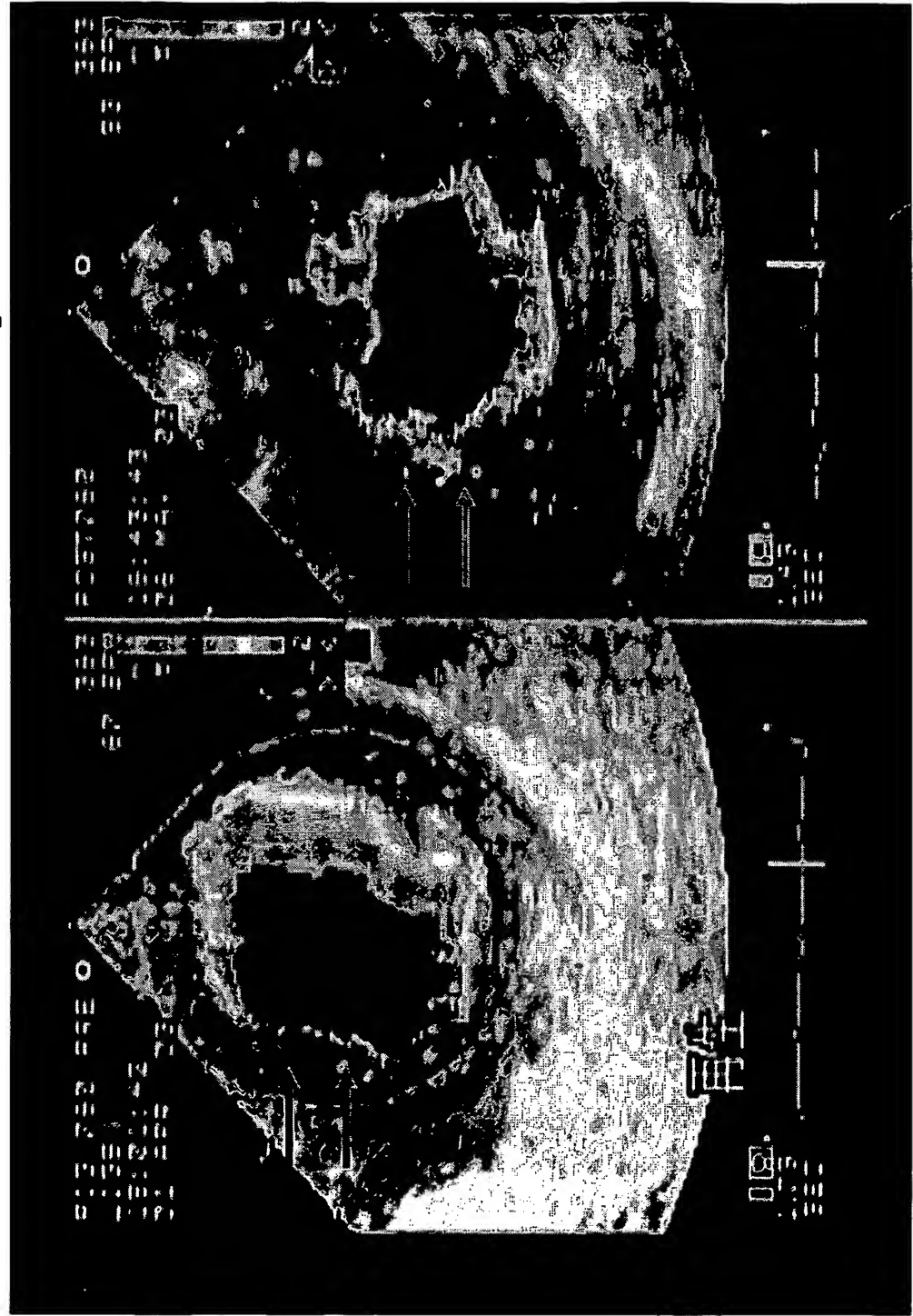


FIG.35
Evaluation of cardiac function (systolic function) of
pig infarction model by CKI method

Before operation

After operation



Implantation
site

Evaluation of cardiac function (diastolic function) of
pig infarction model by CKI method

Before operation After operation

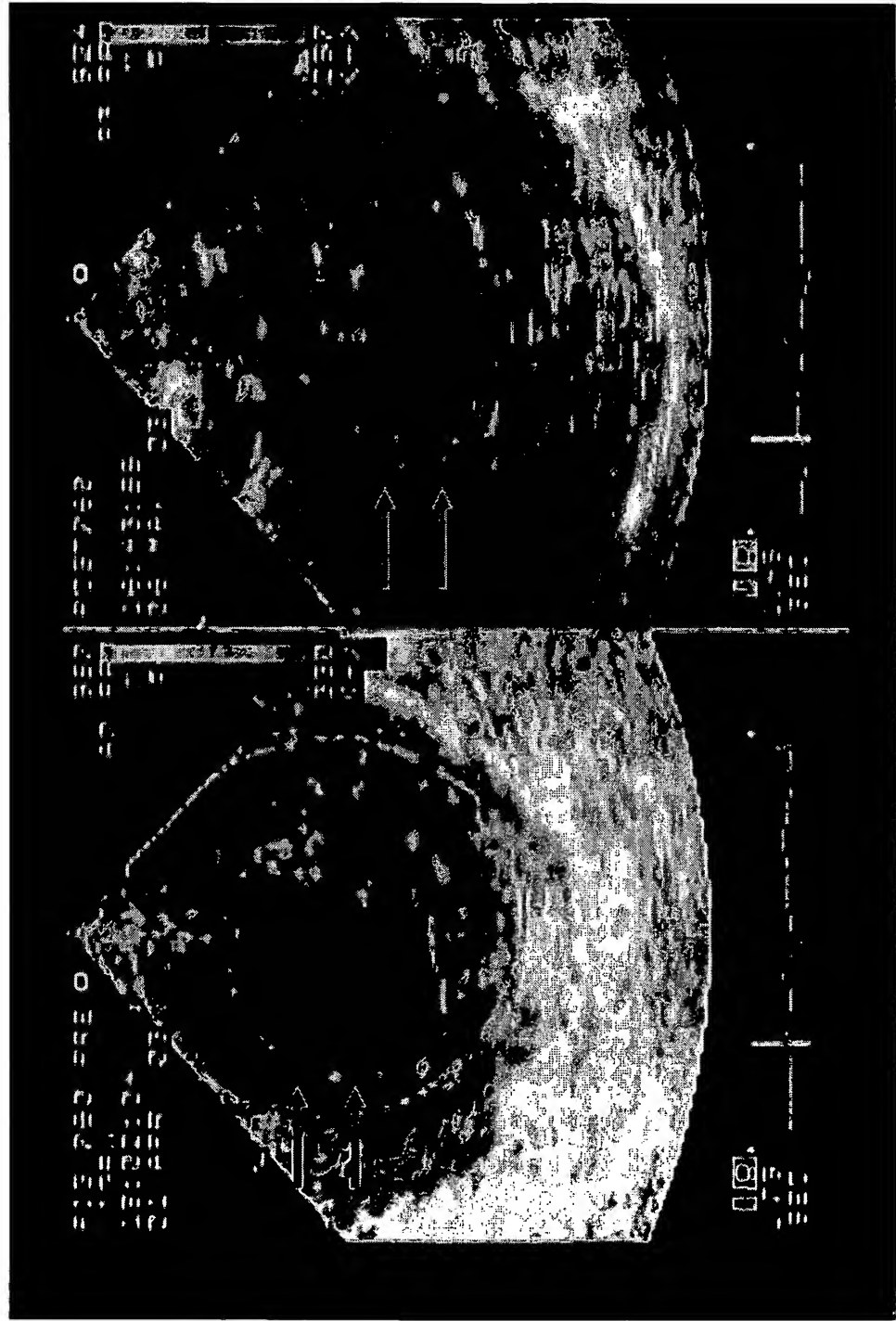


FIG.36

↑
Implantation
site

FIG.37

Without ascorbic acid



FIG.38

With ascorbic acid

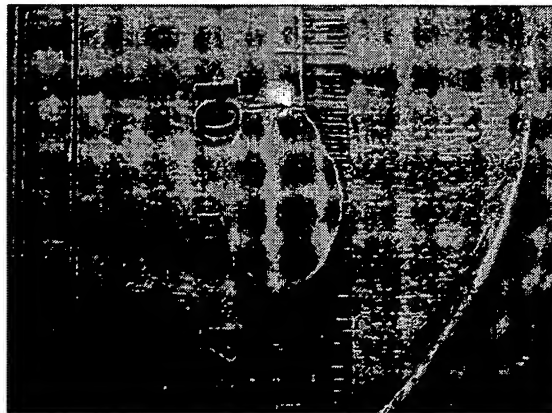


FIG.39

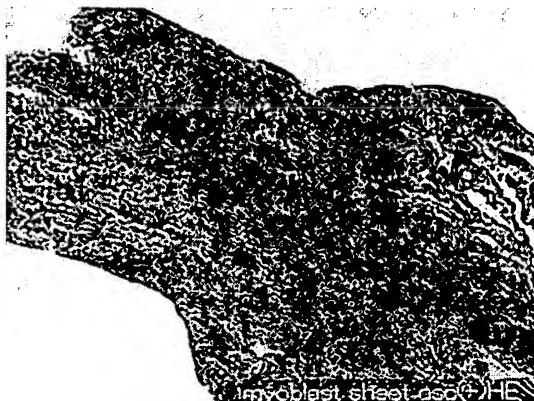


FIG.40

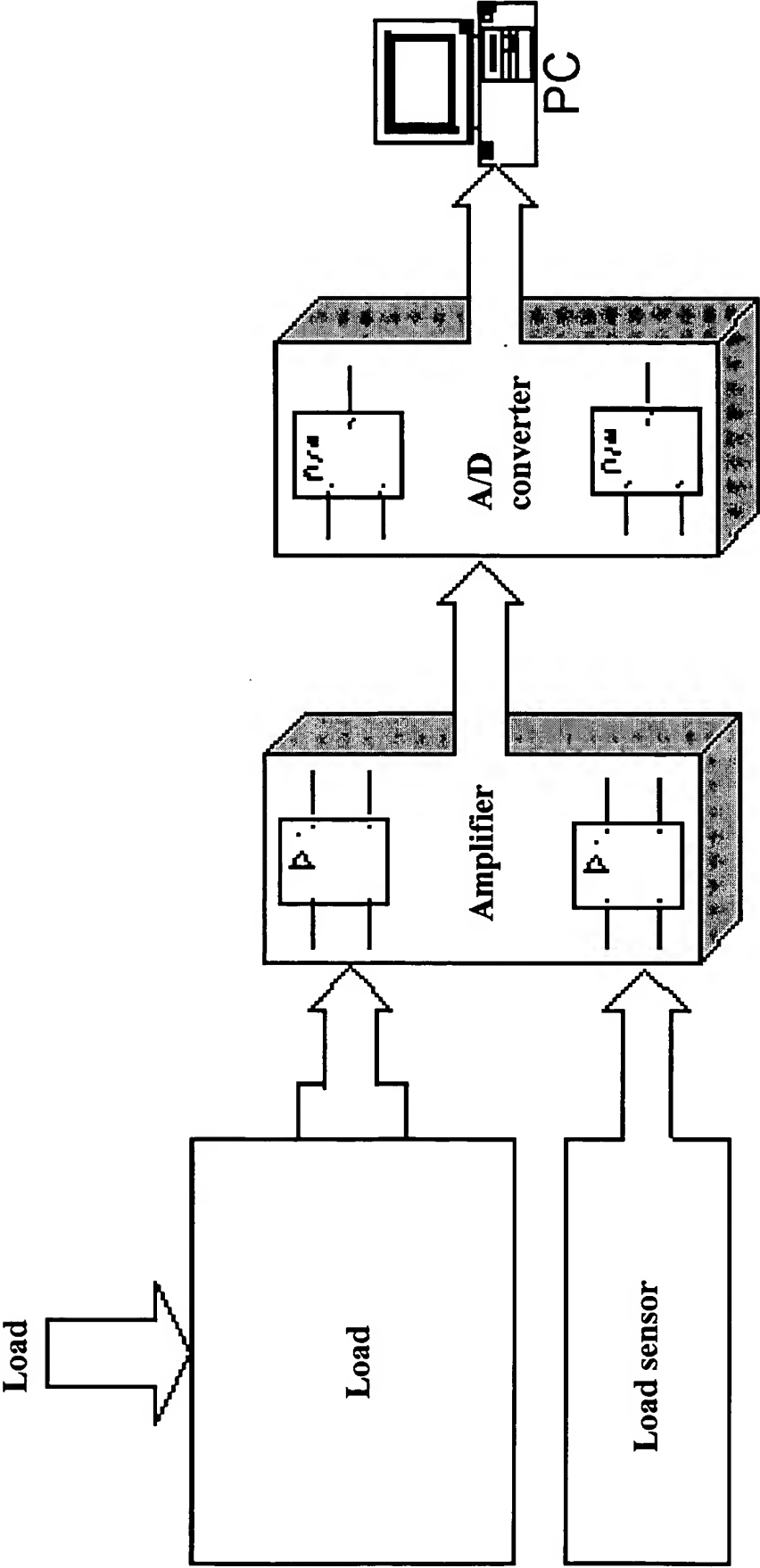
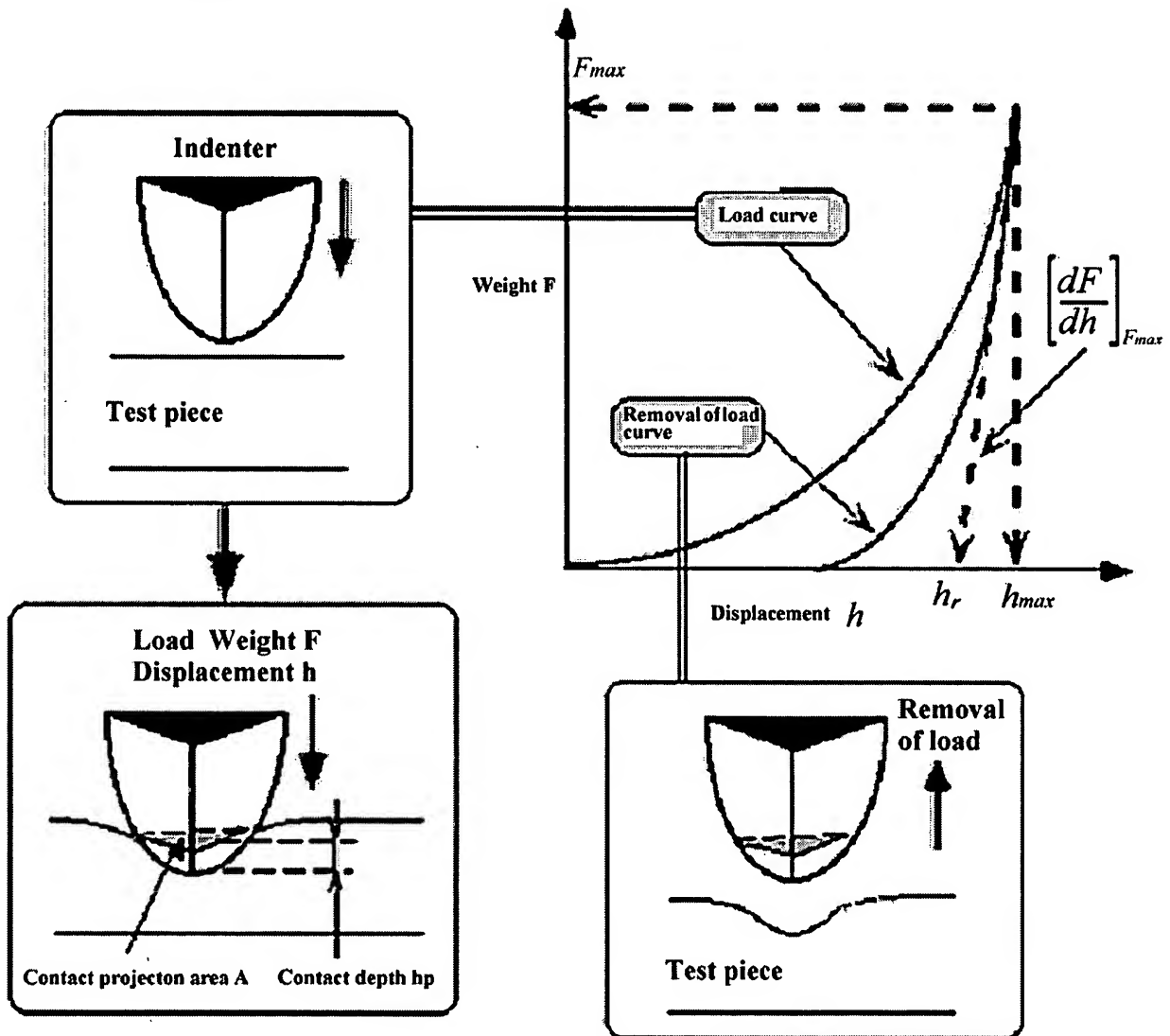


FIG.41



Rigidity $H = \frac{F}{A} = \frac{F}{k_1 h_p^2}$

Young's modulus $E = \left[\frac{dF}{dh} \right]_{F_{max}} \frac{1 - \nu^2}{2 \cdot k_2 \cdot h_{pmax}}$

Contact depth $h_p = h_r + 0.25(h_{max} - h_r)$

F : Load

A : Contact projection area

h_p : Contact depth

k_1, k_2 : Shape coefficient

F_{max} : Maximum load

h_{max} : Max. displacement

h_r : Point at which tangential line cross weight 0

dF/dh : Gradient of tangential line of the removal of load curve

ν : Poisson's ratio

FIG.42



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